LETTERS

Boosting slow oscillations during sleep potentiates memory

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There is compelling evidence that sleep contributes to the long-term consolidation of new memories[1]. This function of sleep has been linked to slow (<1 Hz) potential oscillations, which predominantly arise from the prefrontal neocortex and characterize slow wave sleep[2–4]. However, oscillations in brain potentials are commonly considered to be mere epiphenomena that reflect synchronized activity arising from neuronal networks, which links the membrane and synaptic processes of these neurons in time[5]. Whether brain potentials and their extracellular equivalent have any physiological meaning *per se* is unclear, but can easily be investigated by inducing the extracellular oscillating potential fields of interest[6–8]. Here we show that inducing slow oscillation-like potential fields by transcranial application of oscillating potentials (0.75 Hz) during early nocturnal non-rapid-eye-movement sleep, that is, a period of emerging slow wave sleep, enhances the retention of hippocampus-dependent declarative memories in healthy humans. The slowly oscillating potential stimulation induced an immediate increase in slow wave sleep, endogenous cortical slow oscillations and slow spindle activity in the frontal cortex. Brain stimulation with oscillations at 5 Hz—another frequency band that normally predominates during rapid-eye-movement sleep—decreased slow oscillations and left declarative memory unchanged. Our findings indicate that endogenous slow potential oscillations have a causal role in the sleep-associated consolidation of memory, and that this role is enhanced by field effects in cortical extracellular space.

Slow oscillations reflect widespread 'up' and 'down' states of network activity. These oscillations are generated within the neocortex and are most prominent during slow wave sleep (SWS); the up and down states reflect, respectively, global neuronal depolarization with excitation, and neuronal hyperpolarization with neuronal silence[2,3,9]. Essentially owing to its synchronizing influence on neuronal activity within the neocortex and in dialogue with thalamic and hippocampal circuitry, the slow oscillation has been suspected to underlie the consolidation of memory during sleep[3,10–13]. The slow oscillation signal peaks at 0.7–0.8 Hz, although spectral components can extend into the slow delta band (1–4 Hz)[9,14]. We have examined the role of slow oscillations in memory consolidation by inducing them through transcranial application of oscillating potentials during early nocturnal non-rapid-eye-movement (non-REM) sleep after a learning period.

Hippocampus-dependent declarative memory was assessed by a paired-associate learning task, with memory retention measured by the difference in the number of words recalled when tested after sleep and the number recalled at learning before sleep. As expected from previous studies using the same procedure[8,15], retrieval testing after sleep showed an increase in performance, compared to learning before sleep, in both the slow oscillation stimulation and the sham stimulation conditions (Fig. 1b). However, after slow oscillation stimulation this increase in memory (mean 4.77 words) was greater than that following sham stimulation (mean 2.08 words; $F_{1,12} = 7.96$, $P = 0.01$). In the stimulation condition, 36.50 ± 1.24 words were recalled at learning before sleep, and this number increased to 41.27 ± 1.21 at retrieval after sleep. In the sham condition performance increased from 37.42 ± 0.92 (learning) to 39.50 ± 0.84 words after sleep. This improvement in retention following stimulation is striking considering that most subjects were medical students, who were highly trained in memorizing facts and already performed well in the sham condition. Note that our retention measure does not allow us to differentiate between a slowed decay and an actual gain in memory after stimulation[16].

To test whether slow oscillation stimulation specifically affected the formation of hippocampus-dependent declarative memory, we also tested subjects on a non-declarative, procedural finger-sequence tapping task[17]. Retrieval testing after sleep confirmed the characteristic overnight improvement in skill in both conditions (number of

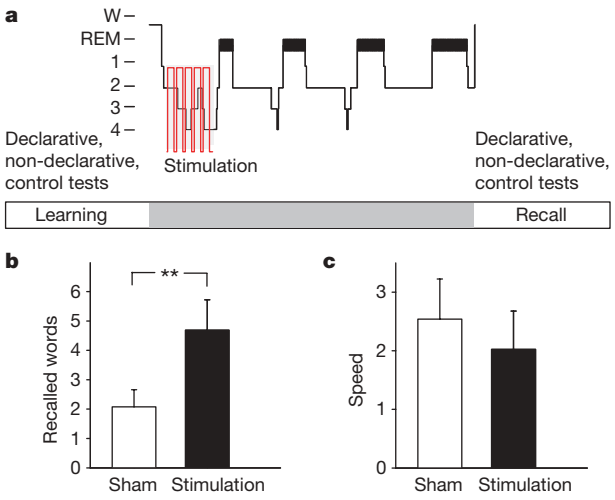

Figure 1 | Slow oscillatory stimulation enhances declarative memory performance. a, Time-course of experiment. Indicated are time points of learning and recall of memory tasks, psychometric control tests, stimulation intervals, period of lights off (horizontal grey bar), and sleep represented by a hypnogram. W, wake; 1–4, sleep stages 1–4. **b**, Performance on the declarative paired-associate memory task across the retention period of nocturnal sleep for stimulation and sham stimulation. Performance is expressed as difference between the number of correct words reported at recall testing and learning. The list contained 46 experimental word-pairs (**$P < 0.01$). **c**, Performance speed on the non-declarative procedural motor skill task across the retention interval expressed as the difference in the number of correctly tapped sequences per 30 s between recall testing and learning. Data are the means ± s.e.m.

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Table 1 | Sleep parameters during transcranial stimulation and sham conditions

Slow oscillation stimulation ($n = 13$)	Stimulation (mean ± SEM)	Sham (mean ± SEM)	Theta stimulation ($n = 5$)	Stimulation (mean ± SEM)	Sham (mean ± SEM)
Awake	0.8 ± 0.8	1.5 ± 1.5	Awake	12.0 ± 12.0	0.0 ± 0.0
S1	17.7 ± 10.3	30.8 ± 12.1	S1	18.2 ± 18.2	24.1 ± 24.1
S2	110.8 ± 14.2	143.1 ± 20.4	S2	181.6 ± 38.0	144.1 ± 30.6
S3	113.1 ± 18.3	85.4 ± 16.2	S3	59.3 ± 34.2	108.1 ± 24.4*
S4	57.7 ± 17.1	39.2 ± 18.7	S4	29.8 ± 13.4	23.9 ± 17.5
SWS	170.8 ± 17.8	124.6 ± 19.2*	SWS	89.1 ± 42.4	132.0 ± 35.0

Time (s) spent in different sleep stages during the 1-min stimulation-free intervals between the periods of stimulation for the main experiment (top) testing slow oscillation stimulation (0.75 Hz) and in a supplementary experiment (bottom) testing theta stimulation (5 Hz). Sleep scoring based on 10-s intervals. Awake and S1 sometimes occurred in just one case. Asterisk indicates a significant ($P < 0.05$) increase in SWS with slow oscillation stimulation and a significant ($P < 0.05$, one-tailed) decrease in S3 sleep with theta stimulation. S1–S4, sleep stages 1–4.

correctly tapped sequences before sleep 17.74 ± 1.30 (stimulation), 18.15 ± 1.28 (sham), and at retrieval after sleep 19.77 ± 1.52 and 20.69 ± 1.46, respectively; $F_{1,12} = 67.70$, $P < 0.001$). However, in contrast to declarative memory performance, the sleep-associated gain in performance (2.03 ± 0.65 sequences) was not enhanced through slow oscillation stimulation ($P > 0.6$; Fig. 1c). Also, overnight changes in error rate did not differ between the two conditions ($P > 0.25$). Performance on two additional tasks, a declarative non-verbal paired-associate task and a procedural mirror-tracing task, likewise indicated that stimulation led to an improvement only for the declarative task (see Supplementary Information for a figure summarizing the main result). The ability of slow oscillation stimulation during early non-REM sleep to enhance retention of word-pairs and its failure to affect procedural skill are consistent with reports that hippocampus-dependent memories benefit mainly from early SWS, and procedural memories from REM sleep (which prevails during late sleep), although non-REM sleep might have complementary functions[1,18,19]. The efficacy of polarization over the prefrontal cortex in our study is in line with this region's importance in the hippocampal–neocortical dialogue that is assumed to underlie the consolidation of hippocampus-dependent memories[20].

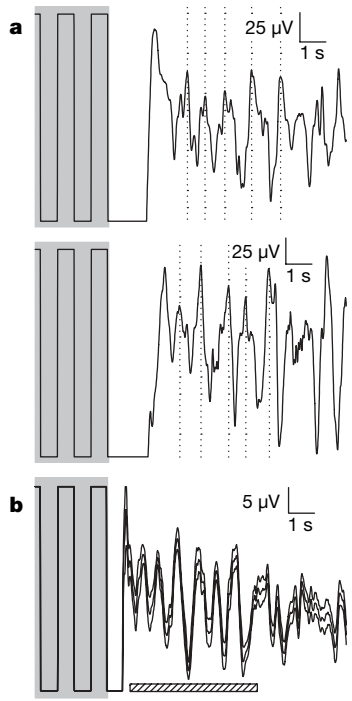

Figure 2 | Synchronization of slow oscillatory EEG activity. a, EEG recordings during the last seconds of a 5-min stimulation period (shaded areas) and first few seconds of a stimulation-free interval of two individuals at prefrontal sites (Fz). **b**, Corresponding mean ±s.e.m. across all subjects and stimulation periods over the parietal cortex (where the EEG is least contaminated by the ceasing stimulation artefact). Positivity upward. Note entrainment of the slow oscillatory EEG activity to the slow oscillatory rhythmic stimulation. Hatched bar indicates time interval of stimulation-induced phase changes in the 0.78–0.98-Hz and 1.37–1.56-Hz bins of the EEG signal.

In a control experiment ($n = 8$) using a protocol identical to that of the main experiment we shifted the timing of stimulation to the period shortly before awakening (05.45–06.15 h), that is closer to retrieval testing, which should increase any immediate non-specific effects of stimulation on cognitive function during retrieval[21]. However, under this condition retention of word-pairs remained unchanged and was similar to retention after sham stimulation (post-sleep retrieval with reference to learning: 3.21 ± 1.43 versus 2.93 ± 1.21 words, $P > 0.8$). These and further control tests of vigilance and general retrieval capabilities (see Supplementary Information) safely exclude any substantial non-specific contribution of slow oscillation stimulation to the improved declarative memory at retrieval testing.

We examined sleep and electroencephalogram (EEG) activity more closely to understand the mechanisms that underlie the enhancement of memory performance. During the 5-min periods of acute stimulation, the induced potentials precluded sleep scoring (Fig. 2). However, the 1-min stimulation-free intervals yielded clear signals. During these intervals, more total time was spent in SWS after slow oscillation (170.77 ± 17.78 s) than after sham stimulation (124.62 ± 19.17 s, $F_{1,12} = 6.03$, $P < 0.05$; Table 1). The times spent in the different sleep stages during the 60 minutes after stimulation and for the whole night were comparable between stimulation and sham conditions (Supplementary Table 1). The increase in SWS is a plausible explanation for the ability of slow oscillatory stimulation to improve memory, particularly as this increase was presumably also present during the periods of acute stimulation. However, visual scoring of SWS relies strongly on an undifferentiated estimation of the presence of slow wave rhythms. We suspected that the transient increase in SWS during the 1-min intervals between periods of stimulation reflected a temporally limited enhancement of EEG slow oscillations, and that this was the specific process that, during this sleep stage, mediated the memory improvement.

Indeed, spectral analysis of EEG activity during the five 1-min intervals between stimulation periods confirmed that stimulation acutely facilitated endogenous slow oscillations. Stimulation distinctly enhanced EEG power within the slow oscillation band (0.5–1.0 Hz, $F_{1,12} = 11.67$, $P < 0.01$ at the frontocentral recording site, Fz; Fig. 3a). A slight increase in power in adjacent low (1–1.5 Hz) delta frequencies and in the 1–4-Hz delta band failed to reach significance ($P > 0.06$, for all comparisons) indicating that the effect of stimulation was focused on the slow oscillation band. Interestingly, slow oscillation stimulation simultaneously enhanced EEG power within the slow spindle frequency range (8–12 Hz, peaking at ~10.5 Hz, $F_{1,12} = 13.12$, $P < 0.01$ at Fz; Fig. 3a) as well as spindle counts (see Supplementary Information). The effects were most pronounced for the first three inter-stimulation intervals (Fig. 3b), with a prefrontal maximum, although they spread to the other recording sites ($F_{1,12} > 7.43$, $P < 0.02$, for overall effects of stimulation). The conjunct increase in slow oscillation and frontal spindle activity agrees well with the notion that neocortical slow oscillations drive the thalamic generation of spindles[3,9,14] and emphasizes that stimulation induces a physiologically coherent pattern of activity in this system.

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Inspection of recordings in some individuals clearly revealed the presence of slow oscillations at the same frequency and in phase with those induced by stimulation, right after stimulation ceased, indicating that the cortical activity boosted by slow oscillation stimulation continued into the stimulation-free period (Fig. 2). In fact, the EEG phase distribution, for a 5-s interval after stimulation ceased, was changed compared to sham stimulation specifically for the frequency bin that approximated the stimulation frequency as well as for the bin that corresponded to twice the stimulation frequency ($P < 0.05$, Kolmogorov-Smirnov; bins 0.78–0.98 Hz and 1.37–1.56 Hz). It has been shown *in vitro* that neuronal networks can synchronize to oscillating electrical fields, showing resonance around particular frequencies[7,22].

We tested the view that resonance specifically in the slow oscillation band is essential for the beneficial effect on memory by using stimulation oscillating at 5 Hz (corresponding to the physiological theta rhythm) under conditions otherwise identical to the main experiment. Compared with sham stimulation, theta stimulation did not increase but rather reduced slow oscillation power in all subjects ($n = 5$) although to variable degrees (at Fz averaged across the first three stimulation-free 1-min intervals: 33.8 ± 14.7 versus $71.5 \pm 20.8\ \mu V^2$ in the sham condition, $P < 0.045$, Wilcoxon). Likewise, theta stimulation in these intervals did not increase, but rather non-significantly decreased slow frontal spindle power (0.60 ± 0.12 versus $0.78 \pm 0.14\ \mu V^2$, $P = 0.34$). Sleep during theta stimulation contained less stage 3 SWS than during sham stimulation (Table 1). Finally, theta stimulation also did not improve declarative memory for word-pairs (post-sleep retrieval with reference to learning: 2.2 ± 1.4 versus 2.4 ± 0.75 words after sham stimulation, $P > 0.5$).

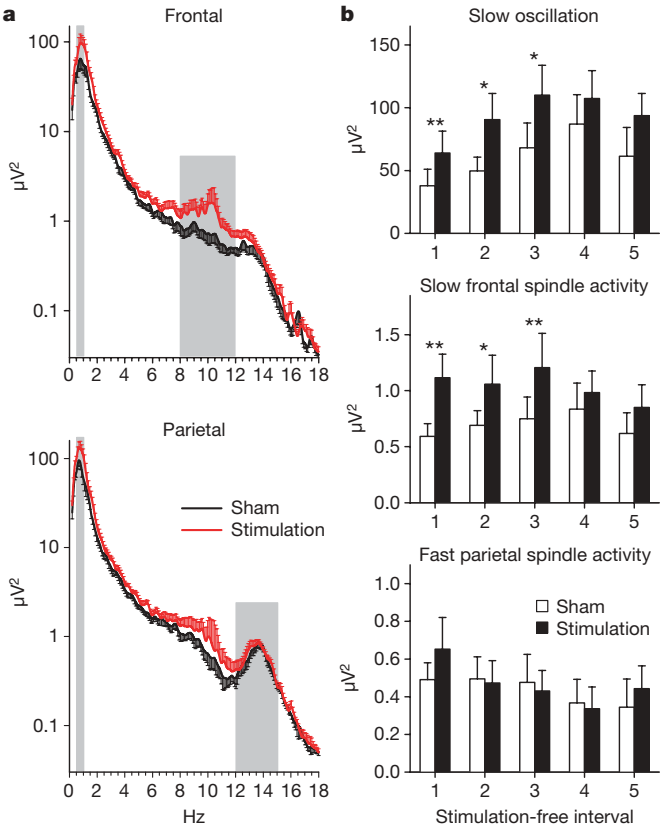

Figure 3 | EEG activity during the 1-min intervals between periods of slow oscillation stimulation and between corresponding periods of sham stimulation. a, Average power spectrum (across first three stimulation-free intervals) at the midline frontal and parietal sites. Shaded areas indicate frequency bands for slow oscillations (0.5–1 Hz), slow frontal spindle activity (upper panel, 8–12 Hz), and fast parietal spindle activity (lower panel, 12–15 Hz). **b**, Time course of power in the five stimulation-free intervals for slow oscillations, slow frontal spindle activity and fast parietal spindle activity. Slow frontal spindle activity is to some extent also visible over the parietal cortex, reflecting the more widespread neuronal synchrony underlying this spindle class[30]. Stimulation enhances slow oscillation and slow spindle activity at the frontal location, but not fast spindle activity at the parietal location. Asterisks indicate statistical significance (**$P < 0.01$, *$P < 0.05$) for pairwise comparison. Data are the means ± s.e.m.

Our results indicate that slow oscillations have a causal role in consolidating hippocampus-dependent memories during sleep. How could slow oscillations promote the plastic neuronal changes that underlie such memory consolidation? One plausible mechanism might involve calcium transients mediated by spindle activity[2,23,24], as spindle activity was enhanced by slow oscillation stimulation. Not only is spindle activity probably associated with massive Ca^{2+} influx into neocortical pyramidal cells, but there is also evidence that repeated spindle-associated spike discharges can trigger long-term potentiation in neocortical synapses[25]. As synchronous spindle activity occurs preferentially at synapses previously potentiated by tetanizing afferent stimulation[26], slow-oscillation-driven spindle activity might contribute to the strengthening of synaptic connections in neocortical circuitry.

Notably, our stimulation induced an estimated potential field in extracellular space that closely resembled that accompanying endogenous slow oscillations. The electric field at the cortical surface right below our stimulation electrode reached an estimated amplitude of ~1,200 $\mu V\ mm^{-1}$ (refs 27, 28). Calculations from local field potential recordings *in vivo* during slow oscillatory activity similarly indicate potential fields up to 1,600 $\mu V\ mm^{-1}$, depending on the distance to the slow oscillation generator (see, for example, ref. 29). Neurons can synchronize to much weaker oscillating fields, as small as 295 $\mu V\ mm^{-1}$ (ref. 22). The synchronizing effect is stronger for neuronal networks than for single neurons, and persists in the absence of functioning chemical synapses[6]. On this background, our results challenge the common view that extracellular slow potential oscillations represent mere epiphenomena without physiological significance *per se*. Because of the insulating property of neuronal membranes, the transcranial stimulation reaches the extracellular space first. Our data therefore indicate that an extracellular slow potential oscillation comparable with that accompanying brain-borne slow oscillations is itself sufficient to increase memory, implicating field effects in cognitive processing during sleep.

METHODS

Oscillating potential fields were induced in young healthy humans ($n = 13$) through stimulation electrodes applied bilaterally at frontolateral locations and at the mastoids, with the frontolateral electrodes representing sites of anodal (positive) polarization. We induced potentials by transcranially applying currents oscillating at a frequency of 0.75 Hz (maximum current density: 0.517 mA cm^{-2}). Stimulation started 4 min after subjects had entered non-REM sleep stage 2 for the first time (without transitions back to stage 1 sleep or wakefulness), that is a time when sleep is expected to progress into SWS. It was applied for five 5-min intervals separated by 1-min intervals free of stimulation. Subjects were tested twice, in a stimulation condition and a sham stimulation condition. In each condition, in the evening before sleep (21.00–22.30 h), subjects learned to a criterion different memory tasks. Recall of memories was tested the following morning (7.00–7.30 h; Fig. 1a). Declarative memory was assessed by a paired-associate learning task[8,15] consisting of a list of 46 word-pairs to be learned before sleep to a criterion of 60% correct responses in a test of immediate cued recall. At retrieval testing in the morning after sleep, cue words were again displayed and the subjects were required to recall the appropriate response word. The procedural task, which has likewise proved sensitive to the enhancing effects of sleep in previous studies[17], required subjects to repeatedly finger-tap with the non-dominant left hand a five-element sequence as fast and accurately as possible on a keyboard for twelve 30-s periods at learning before sleep, and for three 30-s periods at post-sleep retesting. EEG and standard polysomnography were recorded continuously during the sleep period lasting from 23.00 h (lights off) till 6.30 h (awakening; see Supplementary Information for detailed descriptions of the procedure, experimental and analytical techniques).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature. A figure summarizing the main result of this paper is available in Supplementary Information.

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