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Napping to renew learning capacity: enhanced encoding after stimulation of sleep slow oscillations

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Abstract

As well as consolidating memory, sleep has been proposed to serve a second important function for memory, i.e. to free capacities for the learning of new information during succeeding wakefulness. The slow wave activity (SWA) that is a hallmark of slow wave sleep could be involved in both functions. Here, we aimed to demonstrate a causative role for SWA in enhancing the capacity for encoding of information during subsequent wakefulness, using transcranial slow oscillation stimulation (tSOS) oscillating at 0.75 Hz to induce SWA in healthy humans during an afternoon nap. Encoding following the nap was tested for hippocampus-dependent declarative materials (pictures, word pairs, and word lists) and procedural skills (finger sequence tapping). As compared with a sham stimulation control condition, tSOS during the nap enhanced SWA and significantly improved subsequent encoding on all three declarative tasks (picture recognition, cued recall of word pairs, and free recall of word lists), whereas procedural finger sequence tapping skill was not affected. Our results indicate that sleep SWA enhances the capacity for encoding of declarative materials, possibly by down-scaling hippocampal synaptic networks that were potentiated towards saturation during the preceding period of wakefulness.

Introduction

Neocortical slow wave activity (SWA) (0.5–4 Hz), including the < 1-Hz slow oscillations, is a hallmark of slow wave sleep (SWS), and has been shown to play a causal role in the consolidation of declarative memory (Marshall *et al.*, 2006), presumably by driving the redistribution of these hippocampus-dependent memories towards neocortical long-term storage sites (Diekelmann & Born, 2010; Born & Wilhelm, 2011). Apart from this function of consolidating memory, sleep has been proposed to also benefit the encoding of new information during succeeding periods of wakefulness (McDermott *et al.*, 2003; Yoo *et al.*, 2007; Mander *et al.*, 2011). Basically, encoding is an aspect of memory processing that is entirely different from consolidation, and the influences of sleep on both processes are not necessarily linked to a common mechanism. In fact, some findings suggest that the enhancing effects of prior sleep on subsequent encoding during wakefulness originates from rapid eye movement (REM) sleep (Davis *et al.*, 2003; Kim *et al.*, 2005) rather than SWS. However, the preponderance of data appear to support the synaptic down-scaling hypothesis in this context (Tononi & Cirelli, 2003, 2006). This hypothesis assumes that SWS promotes a global down-scaling and desaturation of synapses that were potentiated during encoding of information in the preceding period of wakefulness, and thereby renews the capacity of the network for encoding of further information in the upcoming period of wakefulness. SWA and slow oscillations are considered to play a key role in synaptic down-scaling, because synchronized neuronal firing at this slow rate favours processes of synaptic depression rather than potentiation (Czarnecki *et al.*, 2007). Indeed, a recent study (Van Der Werf *et al.*, 2009) demonstrated that, in elderly individuals, selectively reducing SWA during nocturnal sleep by acoustic stimulation significantly impaired encoding of pictures on the next day. The decrement in learning performance was accompanied by a decrease in hippocampal activity during learning, and both observations were shown to be specific for the encoding of pictures, as procedural learning on a serial reaction time task was not affected by prior suppression of SWA. This pattern, indicating a primary action of SWA on hippocampal encoding of memories, is remarkable, in as much as SWA-dependent synaptic down-scaling is assumed to impact mainly on neocortical networks as the primary source of the slow oscillation (Timofeev *et al.*, 2000; Murphy *et al.*, 2009; Nir *et al.*, 2011), whereas the hippocampus itself does not generate slow oscillations (Isomura *et al.*, 2006). Rather than suppressing SWA, as in the study by Van Der Werf *et al.* (2009), here we aimed to demonstrate a role of SWA in the efficacy of encoding during wakefulness by enhancing SWA through electrical transcranial slow oscillation stimulation (tSOS).

tSOS has proven effective as a means to enhance SWA (Marshall *et al.*, 2006; Kirov *et al.*, 2009). During tSOS, an alternating electric current is applied to the scalp over frontolateral cortical sites with a

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frequency that matches the peak frequency of endogenous slow oscillations (~0.75 Hz) (Steriade *et al.*, 1993; Mölle *et al.*, 2002). The amplitude of the oscillating current stimulation (250 μA) is chosen such that the estimated potential fields in underlying neocortical tissue are about the same size as those that occur naturally during endogenous slow oscillations (Steriade *et al.*, 1996). tSOS applied during non-REM sleep in the first half of the night distinctly increased endogenous slow oscillations and SWA, and this was accompanied by increased frontocortical spindle activity and a significant enhancement in the sleep-dependent consolidation of hippocampus-dependent memory (Marshall *et al.*, 2004, 2006). Animal studies have confirmed that cortical slow oscillation stimulation can effectively synchronize hippocampal activity (Ozen *et al.*, 2010). Here, we hypothesized that applying tSOS during an afternoon nap improves the subsequent encoding of the declarative, i.e. hippocampus-dependent, tasks, with no effect on procedural learning.

Materials and methods

Subjects and procedure

Fifteen subjects aged 23.4 ± 1.9 years (range, 19–27 years; seven women) participated in the experiments. All were right-handed native German speakers with similar educational levels (graduated from German high schools), were non-smokers, were medication-free, and had no history of neurological, mental or sleep disorders. None of them habitually napped during the day. Before the experimental sessions, all subjects were familiarized with the experimental setting by taking an adaption nap in the sleep laboratory (including electrode placement). Subjects who showed no SWS in the adaptation nap were not included in the experiment proper. The experimental protocol was approved by the ethics committee of the University of Lübeck, and the study was conducted in accordance with the 1964 Declaration of Helsinki. All participants gave written informed consent prior to participation.

The experiment proper consisted of two sessions (within-subject cross-over design), balanced in order across subjects, and separated by ~4 weeks (30.75 ± 10.5 days, to diminish carry-over effects between sessions and to control for the female menstrual cycle). In each session, participants were asked to take an afternoon nap and perform on various learning tasks after the nap. In one of the two sessions, tSOS was applied during the nap, whereas in the other session, which served as control, sham stimulation (with an equal set-up but no stimulation) was applied. See Fig. 1A for the experimental procedure.

On experimental days, subjects were required to get up at 05:00 h (to increase sleep propensity), and this was controlled by an Actiwatch 7 (CamNtech, Cambridge, UK) that was attached to the subject's wrist (of the non-dominant hand on the evening before the session at 20:00 h), and by protocols of day-time activities. Subjects arrived at the laboratory at 14:00 h, were prepared for polysomnographic recordings and tSOS, and went to bed at 15:00 h. tSOS (or sham stimulation) began after subjects had attained stable non-REM sleep for the first time after sleep onset (see below for stimulation parameters). Subjects were woken after either one non-REM–REM sleep cycle (i.e. at the end of the first REM sleep phase) or after 90 min of sleep. After a period of 30 min, to allow recovery from sleep inertia, the encoding phase started; this included learning on

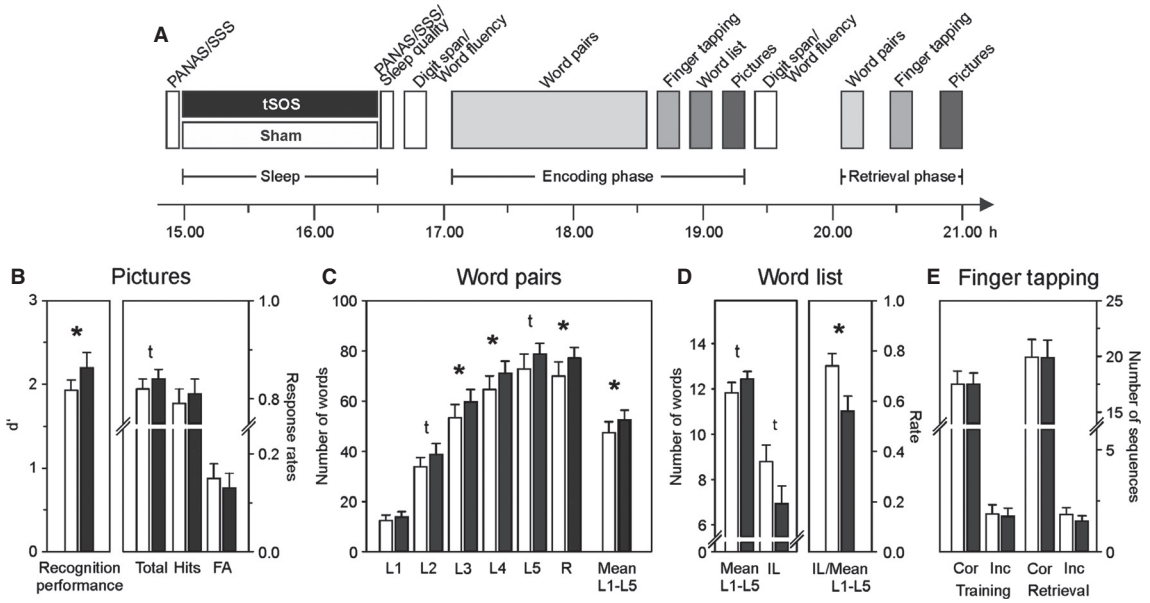

FIG. 1. Study design and learning performance. Black bars: tSOS condition. White bars: sham condition. (A) Subjects took a 90-min nap (15:00 h to 16:30 h), during which either tSOS or sham stimulation was applied. The order of tasks performed during the encoding and retrieval phase is indicated (see text for detailed description of tasks). (B) Learning of pictures. On retrieval testing, subjects showed significantly better recognition of the pictures (*d'*, left) after tSOS during napping than after the sham condition. Right panel: proportions of total correct responses (Total, i.e. hits and correct rejections), hits, and false alarms (FA). (C) Learning of word pairs. tSOS improved recall of word pairs during the five learning trials (L1–L5), and during the delayed retrieval (R). (D) Learning of word list. tSOS tended to increase immediate free recall of words across the five learning runs (Mean L1–L5) and to reduce subsequent learning of the IL. The ratio of IL learning to learning of the original list (IL/Mean L1–L5) was significantly lower after tSOS than after sham stimulation, pointing to enhanced proactive interference after tSOS. (E) Finger sequence tapping. Speed (number of correct sequences; Cor) and accuracy (number of incorrect sequences; Inc) of tapping did not differ between the tSOS and sham conditions at the end of training (10–12th blocks) or during the three blocks of retrieval testing. Means ± standard errors of the mean are shown; $n = 13$ (pictures), $n = 14$ (word pairs), and $n = 15$ (word list, finger tapping). *$P < 0.05$ and t: $P < 0.1$ for pairwise comparison between tSOS and sham stimulation. PANAS, Positive and Negative Affect Scale; SSS, Stanford Sleepiness Scale.

three declarative tasks (pictures, word pairs, and word list) and one procedural task (finger sequence tapping), which always were performed in the same order between 17:00 h and 19:30 h (Fig. 1A). A constant order of tasks was employed to reduce performance variability and because we did not expect any task interactions that would change the direction of tSOS effects. After learning, a standardized meal was served, and this was followed by the retrieval phase ~30 min later.

To control for potential confounding influences of changes in arousal, mood, motivation, and activation, the Positive and Negative Affect Scale (Watson *et al.*, 1988) was applied before sleep and before the encoding phase. To additionally control for potential differences in sleep debt, the Stanford Sleepiness Scale (Hoddes *et al.*, 1973) was applied before and after the nap. Sleep quality during the nap was assessed by use of a questionnaire (Görtelmeyer, 1981). To control for general abilities to retrieve information from long-term memory and for working memory performance and attention, a word fluency task (Aschenbrenner *et al.*, 2000) and the digit span test of the Wechsler Adult Intelligence Scale (Tewes, 1991), respectively, were administered shortly after the nap and after the encoding period. In the word fluency task, participants had to orally generate as many members as possible of a given category (jobs, hobbies, animals, and groceries) and words starting with a given letter (P, K, M, and B) within 2-min intervals. The digit span test consisted of orally presented lists with up to seven digits that the subject had to orally repeat as accurately as possible in the forward and backward directions. Generally, parallel versions of each task were presented in the subject's two experimental sessions.

Learning tasks

All tasks were presented on a computer screen, with E-PRIME 2 (Psychology Tools) and Windows Media Player (for oral presentation of the word lists). The picture learning task was adapted from Van Der Werf *et al.* (2009), and required the subject to encode 50 pictures of landscapes or houses (each presented for 2.5 s), by indicating whether the landscape was tropical or not, or the house was residential or not. Pictures appeared in randomized order with a jittered (0.6–2.4 s) inter-stimulus interval. Responses were given by pressing one of two buttons with the left and right index finger. For retrieval testing, 100 pictures were presented, 50 of which were new and 50 of which had been previously seen. Subjects had to indicate (by button press) whether or not the respective picture occurred during learning, with four possible responses: yes, maybe, maybe not, and no. For analyses, the first two and the last two types of response, respectively, were pooled. The proportions (with reference to the total number of responses) of four response categories were calculated: correctly remembered pictures (hits), correctly rejected, falsely remembered (false alarms), and falsely rejected (misses). As a measure of signal detection performance corrected for response bias, d' was determined for each participant by calculating the z-transformed hit rate minus the z-transformed false alarm rate. Data from two subjects were excluded from analyses of this task; in one case, d' was more than two standard deviations over the mean; in the other, data were missing.

In the word pair learning task, 100 semantically unrelated pairs of German nouns were presented five times. Each pair was presented for 3000 ms (inter-stimulus interval, 500 ms), with one word above the other and a fixation cross in the middle. In each learning trial, word pairs were presented in a different, pre-randomized order. Each of the five trials was immediately followed by a cued recall test, in which the top word (cue) was presented and the bottom word (target) had to be recalled (L1–L5). The order of cue words during each recall was the same as in the foregoing learning trial. Subjects had unlimited time for recall of the target word, and no feedback was provided. An additional recall test took place ~ 90 min after the encoding phase. Data from one subject were discarded, owing to ceiling performance (100% correct).

In the Verbal Learning and Memory Test (the German version of the Rey Auditory Verbal Learning Test) (Helmstaedter *et al.*, 2001), a list of 15 semantically unrelated German nouns was orally presented five times (by a pre-recorded male voice), with each word presented for 1 s. Each presentation was followed by a free recall test (L1–L5). Immediately after the fifth run, a different word list was presented [interference list (IL)], to be recalled. After recall of the IL, participants were asked to again recall the first learnt word list. Individual free recall performance was assessed by calculating the difference between the number of correctly recalled words and the number of incorrect responses (false positives – recalling a word that did not occur in the target list; perseverations – repeating an already given correct response).

In the finger sequence tapping task (Walker *et al.*, 2002), a five-digit sequence (e.g. 4–2–3–1–4) had to be tapped with the four fingers (excluding the thumb) of the non-dominant hand as accurately and as quickly as possible. During learning, subjects performed on 12 30-s blocks with 30-s breaks in between. During retrieval, they performed on three 30-s blocks, similarly to learning. The sequence was presented continuously on a screen. No immediate feedback was given on pressing a key, but, after each block, the number of correct sequences and the total number of tapped sequences were presented.

tSOS

The parameters for tSOS were similar to those in Marshall *et al.* (2006). The stimulating current oscillated between 0 and 250 μA at a frequency of 0.75 Hz. Anodal electrodes (10 mm in diameter) were positioned bilaterally at F3 and F4 (according to the 10–20 system), and reference electrodes were placed at both mastoids. The electrode resistance was < 5 kΩ. The maximum current density at the stimulation sites reached ~0.318 mA/cm^2. tSOS began after 4 min of the first occurrence of continuous non-REM sleep stage 2, and consisted of six to eight 4-min stimulation epochs during non-REM sleep. The number of 4-min stimulation epochs depended on the individual subject's sleep, as we aimed to apply tSOS only during non-REM sleep. Stimulation periods were separated by stimulation-free intervals of at least 1 min. During these stimulation-free intervals, online sleep scoring was performed to ensure that subjects still showed non-REM sleep stage 2 or SWS. If not (that is, the participant was awake or in sleep stage 1), stimulation was delayed until the subject had again entered non-REM sleep stage 2 for 2 min. At the beginning and at the end of each stimulation interval, a trigger was set to mark the stimulation interval. In the sham sessions, electrodes were placed and triggers were set as in the tSOS sessions, but the stimulator remained off. Post-experimental debriefing ensured that subjects were not aware of whether or not they had been stimulated.

Sleep electroencephalogram (EEG) recordings and analyses

The EEG was recorded with Ag/AgCl electrodes placed at Fz, C3, Cz, C4, P3, Pz, and P4, according to the 10–20 system, all referenced to an electrode attached to the nose. The ground electrode was placed on the forehead (Fpz). Electrode impedances were

< 5 kΩ. EEG signals were recorded with a Neurofax EEG-9200 (Nihon Kohden Corporation, Tokyo, Japan), and filtered between 0.05 and 30 Hz. Additionally, horizontal and vertical eye movements and a chin electromyogram were recorded for standard polysomnography and for artefact detection. All recordings were sampled at 500 Hz and stored for later offline analyses. Sleep stages (1, 2, 3, and 4, and REM sleep), wakefulness time and movement artefacts were scored offline for 30-s intervals (Rechtschaffen & Kales, 1968).

Analyses of the acute effects of tSOS on the sleep EEG signal during the 4-min periods of stimulation were focused on the spindle frequency band (9–15 Hz). As several studies have shown that the slow oscillation has a synchronizing effect on spindles, we expected that acute effects of the stimulation would primarily show up in the spindle band frequency, although we also performed exploratory analyses for the faster beta frequency band (15–20 Hz). Because of the strong contamination in the EEG originating from the stimulation signal, which also prevented the standard scoring of sleep stages for these periods, all activity below 4 Hz was removed by means of a digital finite impulse response filter. This analysis was also restricted to the parietal channels (P3, Pz, and P4), because of saturation artefacts in the recorded signals of all other channels caused by the high amplitudes during stimulation. For these 4-min periods, the band-pass-filtered (5–25 Hz) EEG signal was subjected to the calculation of time–frequency plots of wavelet power in a time window ± 2 s around the sine wave peak of the tSOS signal. Additionally, we visually scored and compared arousals during the stimulation and sham stimulation periods by using the electromyogram, vertical and horizontal electrooculogram and EEG in Pz. For both conditions, we applied a 5-Hz high-pass filter on all four signals before scoring of arousals.

In addition to changes occurring during ongoing stimulation, the effects of tSOS on sleep and EEG activity were analysed for 1-min intervals, starting 3 s after the termination of a 4-min period of tSOS or sham stimulation. The analyses concentrated on the first six of these stimulation periods, because this was the minimum number of stimulation periods applied in each subject in both conditions (number of stimulations for sham/tSOS conditions: 6/6 for $n = 2$; 7/7 for $n = 1$; 8/8 for $n = 10$; 7/8 for $n = 1$; 8/6 for $n = 1$). For this analysis, we determined the sleep stage for the first 30-s interval of every stimulation-free period, resulting in one value (between 0 and 4; REM sleep never occurred) for each subject and stimulation-free period. For a more fine-grained analysis, the EEG signal during the 1-min intervals was subjected to fast Fourier transformation (frequency resolution, 0.061 Hz), which was applied to seven overlapping (by 8 s) artefact-free (based on visual inspection) EEG segments of 16.384 s (8192 points × 2 ms). A Hanning window was applied to the segments before calculation of the power spectra. Thereafter, for each 1-min stimulation-free interval, mean power was calculated for the following frequency bands: SWA (0.5–4 Hz), slow spindle activity (9–12 Hz), fast spindle activity (12–15 Hz), and beta activity (15–25 Hz). Note that we prefer to call the 9–12-Hz band 'slow spindle activity' rather than 'alpha' activity, as the latter term is typically used with reference to awake EEG activity. Slow spindle activity during non-REM sleep is clearly concentrated over prefrontal cortical areas, and represents a phenomenon entirely different from the awake alpha activity, which shows parieto-occipital dominance (Anderer *et al.*, 2001; De Gennaro & Ferrara, 2003; Mölle *et al.*, 2011).

To investigate whether spindle activity correlated with memory-encoding measures, discrete fast spindles were detected in Pz, P3 and P4 separately for the stimulation and sham conditions, with an algorithm described elsewhere (Mölle *et al.*, 2011). In brief, EEG data were band-pass-filtered between 12 and 15 Hz, and the root mean square (RMS) was calculated with a moving window of 0.2 s. An amplitude threshold, which was set to 1.5 times the average standard deviation of the band-pass-filtered signal in the three channels, was applied. A spindle was detected if the RMS signal remained suprathreshold for 0.5–3 s. The following spindle activity measures were then calculated as means across the six stimulation epochs and the following stimulation-free intervals: EEG power in the spindle frequency range (12–15 Hz), spindle count, spindle density, spindle peak-to-peak amplitude, spindle RMS amplitude, and spindle length.

Statistical analyses

ANOVAS (SPSS version 19 for Windows) were performed, including the repeated-measures factor 'stimulation' (tSOS vs. sham stimulation). An 'order' factor (tSOS in first vs. second session) was included to explore whether familiarity with the task after an individual's first session influenced performance on the second session. Significant interactions were specified with *post hoc t*-tests. Degrees of freedom were corrected according to Greenhouse–Geisser, where appropriate. The level of significance was set to $P \leq 0.05$.

Results

tSOS during non-REM sleep enhances subsequent encoding in declarative tasks

In the picture learning task, overall encoding of pictures, as indicated by d', was significantly better after the nap when tSOS was applied than after sham stimulation during the nap (2.20 ± 0.18 vs. 1.93 ± 0.12 (mean ± standard error of the mean); $F_{1,12} = 4.82$, $P = 0.048$; Fig. 1B). Corresponding changes in hit rates (i.e. the number of correctly recognized pictures) and in false alarms (i.e. falsely recognized pictures) did not reach significance (see Table 1 for a summary of results). As d' is the most sensitive indicator of encoding, taking into account also the subject's response bias, this pattern basically indicates an enhancing effect of tSOS on encoding of pictures, although this influence appears to be of moderate size, and is masked with measures (such as hit rate) that are confounded by response bias. There was also a tendency for there to be, overall, more correct responses (i.e. hit rate plus correct rejections) and fewer incorrect responses (i.e. false alarms plus misses) after tSOS than after sham stimulation (total rate of correct responses, 0.84 ± 0.02 vs. 0.82 ± 0.02; total rate of incorrect responses, 0.16 ± 0.02 vs. 0.18 ± 0.02; $F_{1,12} = 3.41$, $P = 0.09$; Fig. 1B).

In the word pair learning task, subjects overall learned significantly more word pairs after tSOS than after sham stimulation (mean number of learnt words: 52.40 ± 3.99 vs. 47.41 ± 4.28; $F_{1,12} = 5.07$, $P = 0.044$; Fig. 1C and Table 1). The analyses of word pair recall included an additional factor, 'learning trial' (L1–L5). Learning significantly improved over the five learning trials in both conditions ($F_{4,48} = 316.98$, $P < 0.001$), with the stimulation condition showing better learning performance from the second presentation onwards (L1, $F_{1,12} = 0.38$, $P = 0.561$; L2, $F_{1,12} = 4.36$, $P = 0.059$; L3, $F_{1,12} = 6.15$, $P = 0.029$; L4, $F_{1,12} = 5.21$, $P = 0.041$; L5, $F_{1,12} = 3.42$, $P = 0.089$; Fig. 1C). tSOS also significantly improved cued recall in a delayed retrieval test performed ~90 min after learning (77.14 ± 4.13 vs. 69.93 ± 5.58 after sham stimulation; $F_{1,12} = 6.03$, $P = 0.03$; Fig. 1C).

Analyses of word list recall in the Verbal Learning and Memory Test included an additional factor, 'learning trial'. Mean encoding of

TABLE 1. Learning performance

Learning task	Sham (mean ± SEM)	tSOS (mean ± SEM)	*P*
Pictures			
d′	1.93 ± 0.12	2.20 ± 0.18	0.04*
Hits	0.79 ± 0.03	0.81 ± 0.03	0.28
Correct rejections	0.85 ± 0.03	0.87 ± 0.03	0.33
Total correct responses	0.82 ± 0.02	0.84 ± 0.02	0.09t
False alarms	0.15 ± 0.03	0.13 ± 0.03	0.33
Misses	0.22 ± 0.03	0.19 ± 0.03	0.28
Total incorrect responses	0.18 ± 0.02	0.16 ± 0.02	0.09t
Word pairs (number of words)			
Learning trial 1 (L1)	12.43 ± 2.16	13.79 ± 2.09	0.56
Learning trial 2 (L2)	33.86 ± 3.62	38.71 ± 4.42	0.06t
Learning trial 3 (L3)	53.29 ± 5.33	59.57 ± 5.11	0.03*
Learning trial 4 (L4)	64.64 ± 5.36	71.14 ± 4.67	0.04*
Learning trial 5 (L5)	72.86 ± 5.82	78.79 ± 4.15	0.09t
Delayed retrieval	69.93 ± 5.58	77.14 ± 4.13	0.03*
Mean L1–L5	47.41 ± 4.28	52.40 ± 3.99	0.04*
Word list (number of words)			
Learning trial 1 (L1)	8.40 ± 0.70	8.67 ± 0.49	0.65
Learning trial 2 (L2)	10.67 ± 0.77	11.47 ± 0.49	0.25
Learning trial 3 (L3)	12.67 ± 0.66	13.40 ± 0.51	0.20
Learning trial 4 (L4)	13.53 ± 0.46	14.20 ± 0.30	0.06t
Learning trial 5 (L5)	13.87 ± 0.29	14.47 ± 0.26	0.18
IL	8.80 ± 0.72	6.93 ± 0.79	0.06t
Retrieval (R)	13.87 ± 0.42	14.20 ± 0.26	0.42
Mean L1–L5	11.83 ± 0.45	12.44 ± 0.33	0.07t
IL/mean L1–L5	0.74 ± 0.05	0.56 ± 0.06	0.01*
Finger tapping (number of sequences)			
Training: correct	17.49 ± 1.26	17.18 ± 1.02	0.61
Training: incorrect	1.84 ± 0.46	1.73 ± 0.41	0.78
Retrieval: correct	19.93 ± 1.58	19.87 ± 1.58	0.93
Retrieval: incorrect	1.82 ± 0.34	1.51 ± 0.26	0.30

Mean ± SEM values are indicated for the tSOS and sham conditions for learning of pictures (*d′* and relative frequencies of hits, correct rejections, total correct responses, false alarms, misses, and total incorrect responses), word pairs (number of recalled word pairs of a maximum of 100), and of word lists (number of recalled words from lists of 15 words). *d′* refers to the difference 'hits minus false alarms' (after z-transformation of both values). Word pairs and the word lists were presented five times, with each presentation being followed by an immediate recall (L1–L5). The five presentations of the word list were followed by presentation and recall of an IL; thereafter recall of the originally learnt list was tested (R). The ratio of interference learning to learning of the original list (IL/mean L1–L5) was calculated as measure of proactive interference. Right column: *P*-level for statistical comparison between the tSOS and sham conditions. SEM, standard error of the mean. *$P < 0.05$; t: $P < 0.1$.

the word list across L1–L5 also tended to be enhanced after tSOS, as compared with sham stimulation (number of recalled words: 12.44 ± 0.33 vs. 11.83 ± 0.45; $F_{1,14} = 3.78$, $P = 0.072$; Fig. 1D; see Table 1 for performance during single learning trials). Interestingly, learning of the IL tended to be worse after tSOS than after sham stimulation (6.93 ± 0.79 vs. 8.80 ± 0.72; $F_{1,14} = 4.26$, $P = 0.058$), pointing to stronger proactive interference resulting from enhanced encoding of the list to be learnt first in the tSOS condition. This interpretation was confirmed by calculating the ratio between learning of the IL and the mean learning of the original list (i.e. IL divided by mean L1–L5), which indicated a significantly lower ratio of interference learning after tSOS than after sham stimulation (0.56 ± 0.06 vs. 0.74 ± 0.05; $F_{1,14} = 8.27$, $P = 0.012$).

tSOS during non-REM sleep does not affect procedural skill or performance on control tests

Performance on the finger sequence tapping task, as indicated by the number of correctly tapped sequences per 30-s interval, did not differ between the tSOS and sham conditions, either during training or at retrieval testing (Fig. 1E). In both conditions, subjects equally improved from training to retrieval testing ($F_{1,14} = 13.83$ and $P = 0.002$, for 'training/retrieval' main effect). Performance on the digit span test measuring working memory capacity, and the word fluency test measuring the capability for retrieval from long-term memory, also did not differ between conditions (Table 2).

tSOS increases the depth of non-REM sleep and SWA, and phase-locks 8–20 Hz activity including spindles

Total sleep time was very similar during the tSOS and sham stimulation conditions (74.1 ± 3.3 vs. 76.2 ± 3.4 min; Table 3), and 4-min intervals of (sham) stimulation also occurred equally often (7.60 ± 0.18 vs. 7.53 ± 0.21 intervals; Table 3). In most cases ($n = 13$), subjects were woken after the end of the first REM sleep period. Visual scoring of arousals during the (sham) stimulation periods showed that the number of arousals was, on average, slightly lower during the stimulation condition than during the sham condition (mean ± SEM: 7.27 ± 1.35 vs. 8.93 ± 1.68; $P = 0.16$), but did not significantly differ between the two conditions.

During the 4-min intervals of stimulation, endogenous SWA cannot be separated from the induced tSOS sine wave stimulation

TABLE 2. Control test performance

	Before		After	
Test	Sham (mean ± SEM)	tSOS (mean ± SEM)	Sham (mean ± SEM)	tSOS (mean ± SEM)
Digit span				
Forward	8.53 ± 0.76	8.53 ± 0.43	9.07 ± 0.55	8.53 ± 0.46
Backward	8.67 ± 0.75	9.33 ± 0.52	10.33 ± 0.60	9.67 ± 0.69
Word fluency				
Letters	19.07 ± 1.13	17.53 ± 1.13	25.20 ± 1.36	25.40 ± 1.23
Categories	25.80 ± 1.67	25.53 ± 2.32	41.33 ± 2.91	40.53 ± 2.23
PANAS				
Positive score	2.73 ± 0.14	2.55 ± 0.12	1.87 ± 0.13	1.95 ± 0.16
Negative score	1.00 ± 0.02	1.02 ± 0.03	1.05 ± 0.03	1.10 ± 0.05
SSS	3.00 ± 0.29	3.00 ± 0.17	4.20 ± 0.28	4.27 ± 0.30

'Before' and 'after' refer to before and after learning with regard to digit span and word fluency performance, and to before and after the nap with regard to PANAS and SSS ratings, respectively. Values for PANAS and SSS represent ratings on scales ranging, for the PANAS, from 1 (low) to 5 (high) amounts of positive and negative affect, and, for the SSS, from 1 (low) to 7 (high) amounts of sleepiness. There were no significant differences between the tSOS and sham conditions. PANAS, Positive and Negative Affect Scale; SEM, standard error of the mean; SSS, Stanford Sleepiness Scale.

TABLE 3. Sleep architecture and arousal information

	Sham (mean ± SEM)	tSOS (mean ± SEM)	*P*
Number of stimulations	7.47 ± 0.22	7.53 ± 0.22	0.72
Total stimulation time	31.13 ± 0.92	31.30 ± 0.91	0.79
Total time in bed	77.00 ± 3.41	75.90 ± 3.33	0.78
Total scored sleep time (excluding stimulations)	41.21 ± 2.68	38.77 ± 2.91	0.82
Sleep onset	6.83 ± 1.43	5.83 ± 0.68	0.52
Awake	3.17 ± 0.95	2.03 ± 0.93	0.42
Sleep stage 1	7.93 ± 1.38	5.63 ± 0.84	0.13
Sleep stage 2	12.30 ± 2.11	14.63 ± 1.59	0.42
Sleep stage 3	5.03 ± 1.20	5.53 ± 1.16	0.56
Sleep stage 4	5.30 ± 1.50	6.73 ± 1.72	0.49
REM sleep	5.30 ± 1.24	4.20 ± 0.89	0.47
Number of arousals	25.93 ± 2.98	24.00 ± 2.73	0.47
Sleep efficacy	91.58 ± 2.45	95.38 ± 1.95	0.26

Time is indicated in minutes. Sleep efficacy represents the relative amount of sleep in relation to the total (scored) sleep time in %.

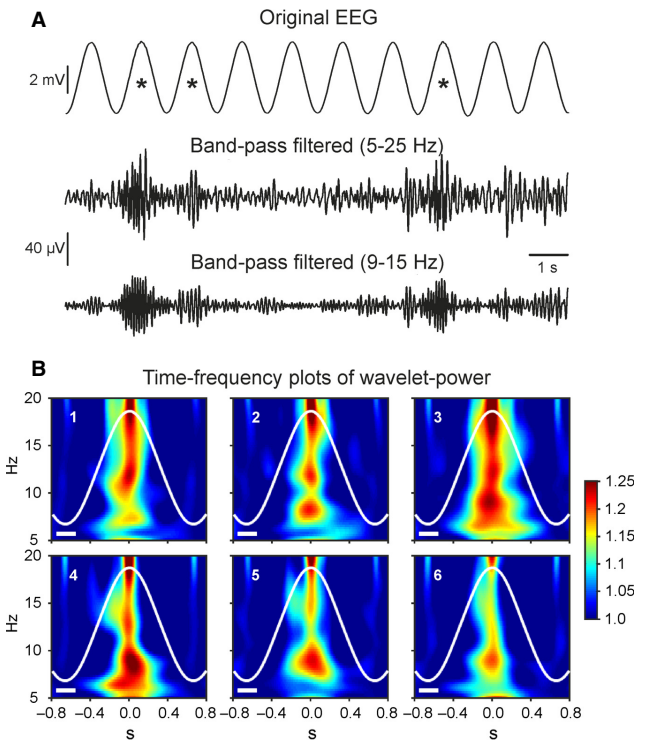

FIG. 2. Acute effects of tSOS on spindle power. (A) A 13.3-s excerpt of EEG at Pz during 10 stimulated tSOS waves. From top to bottom: original EEG, band-pass-filtered (5–25 Hz) EEG used for wavelet analysis, and band-pass-filtered (9–15 Hz) EEG illustrating spindle activity. Asterisks indicate stimulated tSOS waves with enhanced spindle activity around the peak of the sine wave stimulation. (B) Time–frequency plots of wavelet power in Pz ±0.8 s around the peak of tSOS sine waves ($t = 0$) and for frequencies of 5–20 Hz separately for the six stimulation epochs (white number in the upper left corner). Note the strong enhancement in 9–15-Hz spindle wavelet power during the peak of the stimulation (thick white lines indicate averages of the original EEG). Colouring indicates relative wavelet power, with the average power during the minimum stimulation potential (horizontal white line: –0.76 to –0.56 s) set to 1.

signal covering the same frequency band (Fig. 2A). However, after high-pass filtering, an analysis of spindle activity during ongoing stimulation was possible. The corresponding statistical ANOVA included factors representing the stimulation period and the different electrode sites, as well as an additional phase factor (discriminating up-phases and down-phases of the tSOS sine wave signal). In Pz, induction of SWA by tSOS was acutely accompanied by distinct increases in a broad frequency range of 8–20 Hz during the anodal up-phases of the oscillating stimulation, as compared with the down-phases of the stimulation signal ($F_{1,14} = 88.45$ and $P < 0.001$ for the 9–15-Hz frequency band; Fig. 2B). This phase-coupling of EEG activity to the tSOS signal covering both the low 9–12-Hz and high 12–15-Hz spindle frequency bands was, for fast spindle activity, most pronounced during the first and third stimulation periods ($F_{5,70} = 3.82$ and $P = 0.011$ for the phase × stimulation period interaction). Exploratory analyses indicated that this phase-coupling also extended to the faster (15–20 Hz) beta frequency band ($F_{1,14} = 72.0$ and $P < 0.001$ for main effect of phase; $F_{5,70} = 2.61$ and $P = 0.059$, for the phase × stimulation period interaction). There was no systematic difference in EEG power in the slow and fast spindle bands or the adjacent beta band (calculated across the entire periods of acute stimulation) from those in the corresponding periods of the sham condition.

Analyses of the 1-min stimulation-free intervals immediately following the 4-min intervals of tSOS (vs. sham stimulation) included factors representing the stimulation period and, in the case of the EEG power, the different electrode sites. This analysis revealed a clear tSOS-induced increase in SWS. This was shown by a higher value for the standard sleep scoring (Rechtschaffen & Kales, 1968), which was restricted to the first stimulation-free interval (Wilcoxon rank test: $Z = -2.83$; $P = 0.005$) and disappeared during subsequent post-stimulation intervals. A deepening influence of tSOS on non-REM sleep was likewise confirmed by an analysis of EEG power spectra for the 1-min intervals following stimulation. As compared with the corresponding intervals after sham stimulation, tSOS significantly enhanced power (at Fz) in the SWA frequency band in the first three stimulation-free intervals ($F_{1,14} = 10.41$, $P = 0.006$, $F_{1,14} = 4.76$, $P = 0.047$, and $F_{1,14} = 8.06$, $P = 0.013$, respectively; Fig. 3A). Whereas power in the slow (9–12 Hz) and fast (12–15 Hz) spindle bands did not differ between the stimulation conditions, power in the beta band (15–25 Hz) was decreased after stimulation in the first stimulation-free interval ($F_{1,14} = 6.02$, $P = 0.028$; Fig. 3D).

Before correlating spindle activity measures with memory-encoding measures, we analysed whether power in the spindle frequency band and discrete spindles during the six stimulation epochs and the following stimulation-free intervals differed between the stimulation and sham conditions. There were no differences in either spindle power or in counts (in Pz for stimulation vs. sham: 112.33 ± 9.18 vs. 110.93 ± 7.91; $P = 0.84$), density [in Pz (counts/30 s): 2.19 ± 0.18 vs. 2.24 ± 0.15; $P = 0.709$] and length [in Pz (s): 0.91 ± 0.03 vs. 0.94 ± 0.03; $P = 0.353$] of detected spindles. In P3, peak-to-peak and RMS amplitudes of detected spindles were slightly smaller during the stimulation condition than during the sham condition [peak-to-peak (µV), 37.1 ± 1.6 vs. 38.0 ± 1.6, $P = 0.042$; RMS (µV), 9.71 ± 0.43 vs. 9.91 ± 0.43, $P = 0.025$]. However, also in Pz and P4, these two measures did not differ between conditions. No systematic positive correlations between all encoding measures of the different memory tasks and all spindle activity measures emerged. Among all 324 correlations, there was only one significant positive correlation for the stimulation condition [which was in Pz between spindle density and the number of incorrect sequences in the encoding phase of the finger sequence tapping task ($r = 0.532$ and $P = 0.041$, uncorrected for multiple testing)].

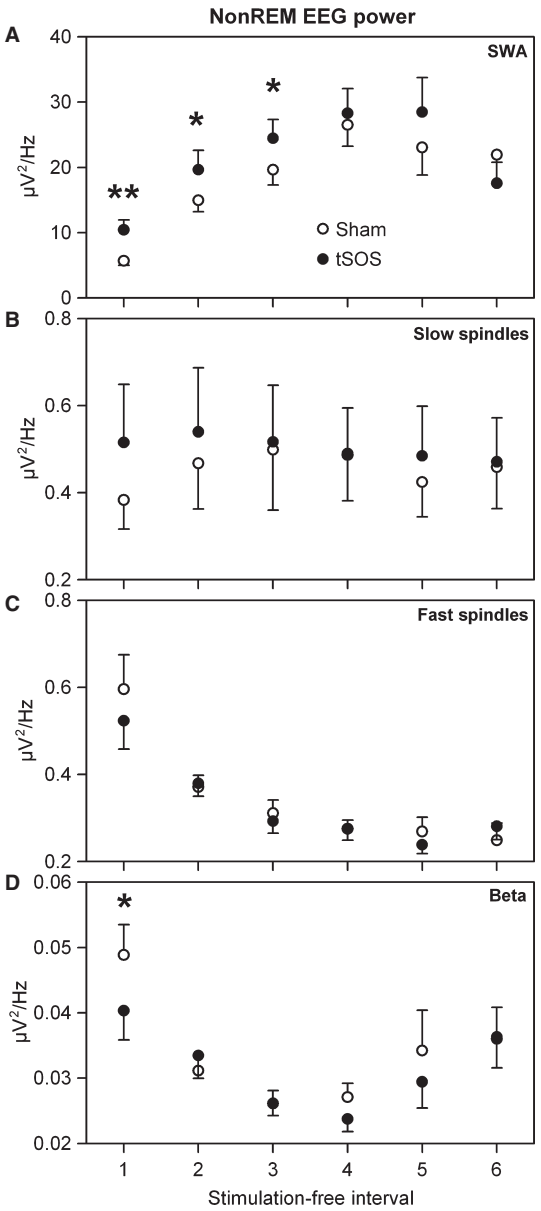

FIG. 3. Effects of tSOS on EEG after acute stimulation. EEG power in a 1-min epoch following stimulation intervals for: (A) SWA (0.5–4-Hz power at Fz); (B) slow spindle activity (9–12-Hz power at Fz), and (C) fast spindle activity (12–15-Hz power at Cz) and beta activity (15–25-Hz power at Cz). Note that SWA is significantly higher following intervals of tSOS than after sham stimulation for the first three 4-min periods of stimulation. Means ± standard errors of the mean are shown. $^{**}P < 0.01$ and $^{*}P < 0.05$ for pairwise comparisons between tSOS and sham stimulation ($n = 15$).

We also analysed how the discrete spindles that were detected during the stimulation epochs were distributed across the phases of the oscillating stimulation. For this purpose, we calculated event correlation histograms of all spindle events (i.e. all peaks and troughs of all detected spindles) across the sine wave of the stimulation signal time-locked to the peak (i.e. maximum stimulation current). This analysis revealed that fast spindle activity was tightly grouped to the up-phases of the oscillating stimulation signal (Fig. 4).

Subjects reported after the nap that they slept more deeply during the tSOS condition than during the sham condition ($F_{1,14} = 6.137$, $P = 0.027$), although ratings of sleepiness (on the Stanford Sleepiness Scale) after the nap did not differ between the stimulation

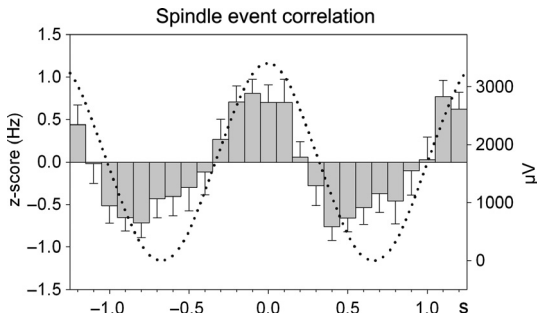

FIG. 4. Temporal association between fast spindles and tSOS. An event correlation histogram of fast spindle activity with reference to the sine wave peak of the stimulation signal (i.e. maximum stimulation current at $t = 0$ s) is shown. Spindle activity (grey bars) was defined by all peaks and troughs of all detected discrete spindles (averaged across P3, Pz, and P4), and z-transformed for each subject. The dotted line represents the original potential recorded from Pz during stimulation averaged across all stimulation sine waves (with reference to the sine wave peak).

conditions ($P = 0.827$, Table 2). Sleepiness also did not differ before the nap ($P = 1$), indicating equal sleep debt in the two conditions. There were also no differences in positive or negative affect (Positive and Negative Affect Scale) between the two stimulation conditions (before nap, positive affect, $P = 0.257$; before nap, negative affect, $P = 0.433$; after nap, positive affect, $P = 0.558$; after nap, negative affect, $P = 0.326$; Table 2). Monitoring of activity (by ActiWatches) did not reveal any difference between the tSOS and sham conditions, confirming that sleep pressure before the nap was similiar between the conditions.

Discussion

The present study demonstrates that tSOS applied during non-REM sleep in an afternoon nap, in comparison with sham stimulation, enhanced subsequent declarative learning of pictures, word pairs, and word lists, whereas training of a procedural finger sequence tapping skill remained unaffected. As expected, tSOS increased the depth of non-REM sleep by increasing SWS and, as a hallmark of SWS, SWA. Acutely, tSOS phase-locked spindle activity to the upstate of the induced slow oscillation. In combination, these findings corroborate and extend previous observations (Van Der Werf *et al.*, 2009) pointing to a causative role of SWA in providing capacities for encoding of new information in the hippocampus-dependent memory system for the upcoming period of wakefulness.

Increase in SWA and depth of non-REM sleep

The application of tSOS oscillating at 0.75 Hz proved to be effective in enhancing SWA and SWS. The effects of tSOS are known to be state-dependent (Steriade *et al.*, 1993; Kanai *et al.*, 2008). Thus, we only applied tSOS when subjects were in non-REM sleep and cortical circuits preferentially resonate in the slow oscillation frequency, which ensured that the effect of tSOS expressed itself mainly as an enhanced SWA. Whereas, during the acute periods of stimulation, endogenous SWA generated in cortical tissue cannot be readily separated from activity in the same frequency band that is related to the stimulation signal, analysis of 1-min periods following the 4-min periods of tSOS confirmed a distinct increase in SWA, especially during the first periods of stimulation. This observation agrees with previous studies (Marshall *et al.*, 2006) in which a similar stimulation protocol conducted during nocturnal sleep enhanced both SWA and SWS during the stimulation-free intervals

immediately after the periods of stimulation, with the effects being also most pronounced during the first three post-stimulation periods.

Considering that, in those previous studies, owing to the strong contamination originating from the stimulation signal, EEG data during actual electrical stimulation could not be analysed, the present study including such analyses of EEG activity during ongoing stimulation represents a clear advance over this previous work. Our time–frequency analyses revealed pronounced phase-coupling of EEG activity in a broad frequency range of 8–20 Hz to the tSOS-induced slow oscillation signal, such that, among activity in other frequency bands, activity in the spindle band culminated during the anodal up-phases of the oscillation. The detection of discrete spindles and the event correlation histograms calculated across all spindle events (peaks and troughs) of all detected spindles clearly showed that those spindles detected during the stimulation were grouped by the up-phases of the oscillating stimulation signal. This observation not only corroborates the acute effectiveness of tSOS, but also strongly supports the conclusion that tSOS-induced SWA does indeed mimic physiologically normal conditions, because, also under natural conditions, endogenous slow oscillations drive spindle generation such that spindles occur preferentially during the slow oscillation up-phase (Mölle *et al.*, 2002, 2011; Steriade, 2003; Steriade & Timofeev, 2003). On the other hand, this finding tempts us to speculate that phase-coupling of spindle activity might secondarily contribute to the enhancing effects of tSOS-induced slow oscillations on encoding. However spindle activity as such is unlikely to be an effective mediator of the enhanced encoding capabilities after tSOS, as spindle activity as such did not differ between the stimulation and sham conditions, and was also not positively correlated in any way with measures of encoding. The fact that induction of slow oscillations by tSOS prevents any direct measurement of endogenous slow oscillations is an obvious limitation of our approach. However, it is of importance in this context that, for tSOS, we chose the maximum current amplitude, such that it induced, in the underlying neocortex, potential fields of a similar amplitude as those naturally observed during SWA, thus closely mimicking endogenous slow oscillations (Steriade *et al.*, 1996). Together, these observations justify the conclusion that the potential fields associated with the occurrence of slow oscillations and SWA do indeed play a causal role in the beneficial effect that these brain oscillations during sleep have on the encoding of information during succeeding wakefulness.

Enhanced learning of declarative but not procedural tasks

The main finding of our study is that tSOS-induced slow oscillation activity during a nap consistently improved subsequent learning on different declarative tasks, whereas training of a procedural skill (finger sequence tapping) was completely unaffected. As training of finger sequence tapping skills is less dependent on hippocampal function than is learning of the declarative tasks, this pattern of findings suggests that SWA particularly benefits encoding in the hippocampus-dependent declarative memory system (Squire *et al.*, 1993; Squire & Zola, 1996; Gais & Born, 2004; Debas *et al.*, 2010). In fact, our pattern of findings is well in line with recent findings by Van Der Werf *et al.* (2009), who tested changes in the learning of declarative and procedural tasks following suppression of SWA during nocturnal sleep by acoustic stimulation. In that study, suppression of SWS, as compared with undisturbed sleep, significantly impaired the encoding of pictures, and this was associated with a significant decrease in hippocampal activation during encoding, whereas training of a finger sequence tapping skill, as in our study, was not influenced by manipulation of SWA. Thus, the results from these two studies are strikingly complementary, although the studies also differed to some extent in their approach and design. Here, we not only enhanced SWA through tSOS, rather than suppressing SWA through acoustic stimulation, but also modified SWA during a single sleep cycle of a nap, rather than during a full night of sleep. Unlike in the study of Van der Werf *et al.*, the encoding period in our study took place immediately after sleep, and retrieval was tested after only a short delay, rather than after another night of sleep. Thus, our procedure enabled a more direct assessment of encoding quality (in the absence of any confounding effects of intervening sleep). Importantly, we show enhancing effects of tSOS-induced SWA not only for the learning and subsequent recognition of pictures, but also for the free and cued recall of learnt verbal materials. Cued and free recall paradigms probe the hippocampal contribution to a memory representation, which basically relies on the forming of new associative connections, to a greater extent than recognition (Tulving & Madigan, 1970; Squire *et al.*, 2007). Thus, the mechanisms and brain regions mediating cued or free recall and recognition differ. Whereas cued and free recall critically rely on a fine-tuned interaction between the prefrontal and hippocampal circuitry, hippocampal contributions to recognition performance are less essential (Mayes *et al.*, 2002; Barbeau *et al.*, 2005; Holdstock *et al.*, 2005; Squire *et al.*, 2007). Hence, our finding that tSOS-enhanced SWA improved the subjects' ability to learn word pairs and word lists as assessed by cued and free recall is another strong hint that the benefit of SWA for encoding of information pertains in particular to the hippocampus-dependent declarative memory system. Along this line of reasoning, there is also evidence from studies in humans and rats that the effects of tSOS on word list learning observed here, indicating an increased susceptibility to proactive interference, likewise reflect basically improved encoding within the prefrontal–hippocampal circuitry (Han *et al.*, 1998; Caplan *et al.*, 2007; Malleret *et al.*, 2010). Thus, rats with neurotoxic lesions to the hippocampus performed better than control rats on a configural learning task specifically when short intertrial intervals were used, because, in this condition, unlike in the controls, performance was not disturbed by proactively interfering response tendencies from the preceding trial (Han *et al.*, 1998). These and related findings suggest that enhanced encoding of events, as observed here after tSOS, may express itself as a transient impairment in encoding similar events, representing enhanced sensitivity to proactive interference. On the other hand, performance on control tests such as the digit span test, which did not indicate any difference between the tSOS and sham stimulation conditions, excluded the possibility that the improved encoding of hippocampus-dependent information after tSOS was secondary to a general improvement in prefrontal working memory function.

The synaptic down-scaling hypothesis is an attractive concept with which to explain our results (Tononi & Cirelli, 2003, 2006; Huber *et al.*, 2007; Massimini *et al.*, 2009). The concept assumes that synaptic connections become globally potentiated, in some cases close to saturation, while information is encoded during wakefulness, and that subsequent SWA during SWS serves to broadly depotentiate and decrease the strength of synaptic connections, thereby renewing the capacity and preparing the synaptic network for the encoding of new information during the following period of wakefulness. As the concept currently concentrates on the homeostatic regulation of synaptic strength within neocortical networks, it does not account for our findings pointing towards a beneficial effect of induced SWA and slow oscillations preferentially on the hippocampal encoding of information. Indeed, we did not observe any improvement in the learning of procedural finger sequence tapping, which is a task relying more on corticostriatal than hippocampal cir-

cuitry (Squire *et al.*, 1993; Squire & Zola, 1996; Debas *et al.*, 2010). Although the hippocampus itself does not generate slow oscillations, it is reached by neocortically generated slow oscillations synchronizing hippocampal with neocortical activity (Sirota & Buzsaki, 2005; Isomura *et al.*, 2006; Clemens *et al.*, 2007; Mölle *et al.*, 2009; Nir *et al.*, 2011). Changes in membrane potentials of hippocampal interneurons are phase-locked to the neocortical slow oscillation, with the synchronizing influence of the neocortical slow oscillation probably being mediated via the temporo-ammonic pathway (Hahn *et al.*, 2006; Wolansky *et al.*, 2006). On this background, our findings tempt us to conclude that SWA and slow oscillations spreading from their neocortical origin down-scale synapses predominantly in the hippocampal circuitry, perhaps because of the generally greater synaptic plasticity of hippocampal than of neocortical networks, although, on the basis of the available data, this conclusion remains tentative. Alternatively, the fact that tSOS specifically improves declarative but not procedural encoding might be attributed to synaptic down-scaling within neocortical networks, whereby tSOS, owing to the positioning of the stimulation electrodes, might have predominantly affected anterior rather than posterior cortical regions. However, this interpretation is unlikely, because procedural learning tasks such as finger sequence tapping also essentially involve frontal cortical areas (e.g. Heun *et al.*, 2004; Fischer *et al.*, 2005). Thus, if tSOS had induced synaptic down-scaling mainly in anterior neocortical networks, this should have also improved learning on the finger sequence tapping task. Slow oscillations support the long-term consolidation of hippocampal memories, presumably by driving the neuronal replay and redistribution of newly encoded hippocampal representations towards neocortical sites of long-term storage (Marshall *et al.*, 2006; Ji & Wilson, 2007; Diekelmann & Born, 2010). The present data suggest that the down-scaling and memory-consolidating actions of slow oscillations in the hippocampus are linked, such that the slow oscillation-induced reactivation and redistribution of recently encoded memories results in a freeing of hippocampal capacities for the encoding of new information.

It is known that sleep and, particularly, SWS facilitate consolidation of hippocampus-dependent declarative memories. In addition, findings after sleep deprivation have pointed to a 'forward' role of sleep in promoting the learning of new materials during subsequent wakefulness (McDermott *et al.*, 2003; Yoo *et al.*, 2007). The involvement of SWA was indicated by a recent study revealing impaired encoding of declarative memories after suppression of SWA (Van Der Werf *et al.*, 2009). In contrast, our study demonstrates a direct enhancing effect of tSOS-induced SWA on the encoding of declarative memory. In combination, these findings corroborate a causal link between sleep SWA and the renewal of hippocampal encoding capacities. Because procedural learning did not benefit from enhanced SWA, SWA-dependent renewal of encoding capacities and the putative underlying processes of synaptic down-scaling appear to predominantly impact on hippocampal networks.

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Abbreviations

EEG, Electroencephalogram; IL, interference list; REM, rapid eye movement; RMS, root mean square; SWA, slow wave activity; SWS, slow wave sleep; tSOS, transcranial slow oscillation stimulation.

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