Slow oscillation electrical brain stimulation during waking promotes EEG theta activity and memory encoding

Roumen Kirov1,3, Carsten Weiss2, Hartwig R. Siebner2,4, Jan Born5, and Lisa Marshall1,6

1Department of Neuroendocrinology, University of Lübeck, 23538 Lübeck, Germany; 2Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria; 3Department of Neurology, Christian Albrechts University of Kiel, 24105 Kiel, Germany; and 4Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital, DK-2650 Hvidovre, Denmark

Edited by Thomas D. Albright, The Salk Institute for Biological Studies, La Jolla, CA, and approved July 24, 2009 (received for review April 23, 2009)

The application of transcranial slow oscillation stimulation (tSOS; 0.75 Hz) was previously shown to enhance widespread endogenous EEG slow oscillatory activity when applied during a sleep period characterized by emerging endogenous slow oscillatory activity. Processes of memory consolidation typically occurring during this state of sleep were also enhanced. Here, we show that the same tSOS applied in the waking brain also induced an increase in endogenous EEG slow oscillations (0.4–1.2 Hz), although in a topographically restricted fashion. Applied during wakefulness tSOS, additionally, resulted in a marked and widespread increase in EEG theta (4–8 Hz) activity. During wake, tSOS did not enhance consolidation of memories when applied after learning, but improved encoding of hippocampus-dependent memories when applied during learning. We conclude that the EEG frequency and related memory processes induced by tSOS critically depend on brain state. In response to tSOS during wakefulness the brain transposes stimulation by responding preferentially with theta oscillations and facilitated encoding.

Transcranial slow oscillation stimulation | sleep | plasticity | cortex | tDCS (transcranial direct current stimulation)

The formation of long-term memories encompasses the stages of encoding and subsequent consolidation, which are linked to separate brain states. Whereas encoding takes place during wakefulness and, for hippocampus-dependent declarative memories, is associated with increased EEG theta activity (1–7), consolidation of such memories appears to be most effectively established “offline” during slow wave sleep (SWS) (8–10), a state that is closely linked to the appearance of EEG slow oscillations (11–13). Slow oscillations are global up and down states of neocortical activity with a peak frequency of ~0.75 Hz in humans (11–16). A recent study using anodal transcranial slow oscillation stimulation (tSOS) at 0.75 Hz (17) to induce slow oscillatory activity in underlying neocortical tissue during non-rapid eye movement sleep in healthy humans provided direct evidence for a causative role of slow oscillation potential fields for the consolidation of hippocampus-dependent memories. In contrast, application of anodal transcranial oscillatory stimulation at theta frequency (5 Hz) failed to produce these effects. Thus, the study demonstrated one possible mechanism through which declarative memory consolidation during sleep is coupled to the endogenously generated slow oscillations characterizing SWS (18).

The objective of the present study was to test whether tSOS induced slow oscillations reflect a mechanism for memory consolidation that is specific for SWS, or whether induced slow oscillatory activity may also subserve memory during wakefulness. One hypothesis is that externally imposed neural dynamics resemble endogenous activity during SWS and may activate processes that internally support memory consolidation beyond sleep. Alternatively, the enhancing effect of tSOS and associated EEG changes on memory consolidation may depend on the functional brain state (19). In a series of experiments during wake, we tested whether tSOS would influence the generation of slow oscillations in the waking brain and likewise improve memory consolidation. The associated changes in EEG activity were taken as a measure of the preparedness of the brain to resonate in a certain frequency.

Results

Transcranial SOS During Quiet Wake Produces a Local Increase in Slow Oscillations and Widespread Increase in Theta and Beta EEG Activities. In Exp. 1 (Fig. S1), tSOS (at 0.75 Hz) induced distinct changes in EEG activity (as assessed during the 1-min stimulation-free intervals after each of the 5-min intervals of stimulation). EEG power in the slow oscillation frequency band (0.4–1.2 Hz) was distinctly increased; however, clearly restricted to the electrode sites closest to the location of the stimulating electrodes, i.e., at the frontal leads F7, Fz, and F8 (stimulation × lead: F10.150 = 4.03, P < 0.01; P < 0.05 for post hoc comparisons at respective electrode sites) (Figs. 1A and 3A). Also, the effect seemed to decrease already at the 5th stimulation period (Fig. 1C). Stimulation produced a most pronounced increase in power in the theta frequency band (4–8 Hz; stimulation: F1.15 = 34.45, P < 0.001; Figs. 1B and 3A). Notably, these effects were equally distributed across electrode sites (stimulation × lead: F10.150 = 0.73, P > 0.5). Transcranial SOS also increased beta activity (15–25 Hz; stimulation: F1.15 = 21.47, P < 0.001) (Fig. 3A). For frontal slow oscillation activity, theta and beta frequencies, power was specifically increased during the 1-min stimulation-free intervals after the five stimulation intervals, but not at 30 or 60 min after the stimulation period (stimulation × time: frontal slow oscillation, F7.105 = 1.99, P = 0.11; theta, F7.105 = 10.18, P < 0.001; beta, F7.105 = 4.89, P = 0.005) (Fig. 1C and D; Fig. S2). All other frequency bands (i.e., delta, slow and fast alpha) were not consistently influenced.

Transcranial SOS During Quiet Wake Does Not Consolidate Memories. Performance at learning was comparable between conditions for the four memory tasks [verbal paired-associate learning, non-verbal paired-associate learning, mirror tracing, and finger sequence tapping (P > 0.4; Table 1)]. Retention of these memories across the 7-h wake interval was not affected by tSOS, as expressed by the differences in performance at retrieval testing and learning (P > 0.3), except for a trend toward enhanced

Author contributions: J.B. and L.M. designed research; R.K. and C.W. performed research; C.W. and H.R.S. contributed new reagents/analytic tools; R.K., C.W., H.R.S., and L.M. analyzed data; and R.K., J.B., and L.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

1To whom correspondence should be addressed. E-mail: marshall@kfg.uni-luebeck.de.

This article contains supporting information online at www.pnas.org/cgi/content/full/0904438106DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.0904438106
Mirror tracing accuracy (no. of errors) 22.78
Mirror tracing speed (s) 78.37

Locations, i.e., averaged across F7, Fz, and F8 (stimulation after termination of stimulation in the slow oscillation band at prefrontal intervals immediately succeeding the stimulation intervals and 30 and 60 min C and B between conditions (tests), self-reported mood, and activation also did not differ working memory and retrieval function (digit span, word fluency stimulation are reported in vals of stimulation in the slow oscillation (0.4–1.2 Hz) (A), and theta (4–8 Hz) A/H11006 F/H11005 Kirov et al. PNAS.

Test (VLMT) was increased significantly by tSOS, mainly during encoding of declarative tasks.

Common EEG Effects Are Produced by tSOS in Attentive and Quiet Wakefulness. The Supplementary experiment was performed to assure that EEG changes induced by tSOS during attentive wakefulness, i.e., during encoding of declarative memories in Exp. 2, were comparable with those revealed during quiet wakefulness in Exp. 1. Indeed, like during quiet wakefulness, tSOS during learning of word lists induced a significant increase in slow oscillation power (0.4–1.2 Hz) during the 1-min intervals later presentation of the list and during free recall after the interference list (IL) (stimulation: $F_{1,11} = 5.49, P < 0.05$) (Fig. 2). In particular, immediate free recalls of the standard list on the 5th and on the 6th trials (the latter after presentation of the IL) were significantly improved by tSOS compared with sham ($F_{1,11} > 6.22, P < 0.03$). In light of recent findings (20, 21) of effects of transcranial stimulation on the rate of false memories, we analyzed aside from total errors also the number of falsely recalled words (i.e., words not in the standard list), preservation type (repetitions) errors, and accuracy (ratio of total correct words by total cited words), but did not find any differences between the sham and tSOS conditions (each $P > 0.1$) (Table 2; Table S2).

Table 1. Performance during learning and retention of memories during sham and stimulation sessions ($n = 16$; mean ± SEM)

<table>
<thead>
<tr>
<th>Memory task</th>
<th>Sham Learning</th>
<th>Stimulation Learning</th>
<th>Sham Retention</th>
<th>Stimulation Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal paired-associates (recalled word-pairs)</td>
<td>41.31 ± 0.76</td>
<td>42.18 ± 0.81</td>
<td>-0.5 ± 0.82</td>
<td>-0.56 ± 0.92</td>
</tr>
<tr>
<td>Non-verbal paired-associates (recalled drawing-pairs)</td>
<td>11.13 ± 0.27</td>
<td>11.44 ± 0.42</td>
<td>-0.75 ± 0.53</td>
<td>-1.44 ± 0.56</td>
</tr>
<tr>
<td>Sequence tapping-speed (no. of sequences)</td>
<td>18.79 ± 1.53</td>
<td>18.21 ± 1.18</td>
<td>1.52 ± 0.38</td>
<td>2.10 ± 1.08</td>
</tr>
<tr>
<td>Sequence tapping-accuracy (no. of errors)</td>
<td>0.81 ± 0.16</td>
<td>1.02 ± 0.22</td>
<td>0.4 ± 0.38</td>
<td>0.01 ± 0.34</td>
</tr>
<tr>
<td>Mirror tracing-speed (s)</td>
<td>78.37 ± 4.53</td>
<td>80.80 ± 4.80</td>
<td>-9.86 ± 2.16</td>
<td>-16.81 ± 2.51</td>
</tr>
<tr>
<td>Mirror tracing-accuracy (no. of errors)</td>
<td>22.78 ± 3.06</td>
<td>23.56 ± 2.71</td>
<td>5.69 ± 1.67</td>
<td>6.84 ± 3.36</td>
</tr>
</tbody>
</table>

Retention is defined by the difference in performance at retrieval testing minus performance at learning. There were no significant differences in retention between the stimulation and sham condition.
after each of the five stimulation intervals, with this increase again restricted to sites closest to the stimulating electrodes, i.e., at frontal locations F7, Fz, and F8 (stimulation \times lead: F_{1,120} = 2.49, P < 0.05; P < 0.05 for post hoc comparisons at these leads) (Fig. 3B). Notably, tSOS was also accompanied by a prominent and widespread increase in theta (4–8 Hz) activity distributed almost equally across electrode sites (stimulation main effect: F_{1,12} = 12.71, P < 0.005; Fig. 3B) (stimulation \times lead: F_{1,120} = 2.10, P > 0.05). During quiet wakefulness, tSOS did not modify beta activity (15–25 Hz; stimulation: F_{1,12} = 0.32, P > 0.5). Both slow oscillation power and beta band activity were increased only during the five stimulation-free intervals, but not at 30 or 60 min after the stimulation period (stimulation \times time, frontal slow oscillation, F_{7,84} = 1.84, P < 0.1, theta, F_{7,84} = 7.82, P < 0.001) (data not shown). All other frequency bands were not significantly influenced.

Discussion

The application of tSOS during a wake retention interval (i) enhanced frontal EEG slow oscillation activity, but was otherwise associated with distinctly different effects than those observed previously after tSOS during sleep (18); (ii) tSOS during quiet and attentive wakefulness enhanced global activity in the theta (4–8 Hz) frequency band [beta activity (15–25 Hz) was additionally enhanced during quiet wakefulness]; (iii) retention of declarative memories during the wake retention interval was not improved; (iv) instead, tSOS during the process of encoding improved learning performance as assessed by immediate recall of words learned in the presence of stimulation. Our findings indicate that the effects of tSOS on memory function critically depend on the functional brain state (19, 25). The presence of slowly oscillating potential fields restricted to prefrontal cortical tissue does not support memory consolidation beyond sleep. Instead, tSOS during wakefulness promotes memory encoding presumably by globally increasing theta EEG oscillations.

Table 2. Performance measures in experiment 2 (n = 12; mean ± SEM)

<table>
<thead>
<tr>
<th>Test</th>
<th>Sham</th>
<th>Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLM, errors</td>
<td>0.33 ± 0.19</td>
<td>0.17 ± 0.11</td>
</tr>
<tr>
<td>R1 (standard list)</td>
<td>0.83 ± 0.24</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td>R2</td>
<td>0.92 ± 0.31</td>
<td>0.42 ± 0.19</td>
</tr>
<tr>
<td>R3</td>
<td>0.58 ± 0.26</td>
<td>0.92 ± 0.34</td>
</tr>
<tr>
<td>R4</td>
<td>0.58 ± 0.26</td>
<td>0.33 ± 0.19</td>
</tr>
<tr>
<td>IL</td>
<td>0.17 ± 0.11</td>
<td>0.67 ± 0.36</td>
</tr>
<tr>
<td>R6</td>
<td>0.33 ± 0.26</td>
<td>0.17 ± 0.11</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>0.75 ± 0.28</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td>No. list learning, recognized nos.</td>
<td>83.6 ± 3.1</td>
<td>82.6 ± 3.1</td>
</tr>
<tr>
<td>List 1, nos.; %</td>
<td>83.3 ± 2.7</td>
<td>79.9 ± 4.7</td>
</tr>
<tr>
<td>Psychometric tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit span forward (digits)</td>
<td>8.16 ± 0.60</td>
<td>8.92 ± 0.69</td>
</tr>
<tr>
<td>Digit span backward (digits)</td>
<td>7.58 ± 0.62</td>
<td>8.00 ± 0.79</td>
</tr>
<tr>
<td>Control tests, before learning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stanford Sleepiness Scale</td>
<td>2.25 ± 0.21</td>
<td>2.08 ± 0.34</td>
</tr>
<tr>
<td>PANAS, positive score</td>
<td>4.61 ± 0.68</td>
<td>4.82 ± 0.82</td>
</tr>
<tr>
<td>PANAS, negative score</td>
<td>2.88 ± 0.56</td>
<td>3.02 ± 0.62</td>
</tr>
<tr>
<td>Control tests, after learning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stanford Sleepiness Scale</td>
<td>2.25 ± 0.30</td>
<td>2.08 ± 0.33</td>
</tr>
<tr>
<td>PANAS, positive score</td>
<td>4.21 ± 0.51</td>
<td>4.57 ± 0.65</td>
</tr>
<tr>
<td>PANAS, negative score</td>
<td>2.46 ± 0.40</td>
<td>3.06 ± 0.64</td>
</tr>
</tbody>
</table>

For all measures, sham vs. stimulation comparisons were nonsignificant.

Increased EEG Slow Oscillation Activity. The ability of tSOS to enhance slow oscillatory activity even during wakefulness in the prefrontal cortex, which is the preferential source of the endogenous sleep slow oscillation (26, 27), may indicate increased frontocortical susceptibility to electric field fluctuations in this frequency. It has been suggested that the initiation of the slow oscillation results from a focus of increased excitability arising from the coincidence of spontaneously occurring miniature depolarizing events (28). This activity may then spread to neighboring neurons; thus, incorporating previously silent cells into an emergent oscillating network. Our tSOS appears sufficient to induce an avalanche response measurable during a short time period in the immediate aftermath of stimulation.

The amplitude of the slow oscillations is thought to reflect global synaptic strength in underlying circuitry with stronger synaptic connectivity resulting in enhanced oscillations with steeper wave slopes (29–31). Global synaptic strength in underlying cortical networks may be low in the beginning of the wake phase, i.e., the time the experiments were conducted, in comparison with conditions in the evening when synaptic potentiation has accumulated over the day (32). On this background, the stimulation-induced increase in slow oscillation activity would be

Fig. 3. Comparative changes in EEG spectral power. tSOS (at 0.75 Hz) induced changes in subsequent EEG spectral power during quiet (A) and attentive (B) wakefulness. EEG power spectra are averages across the five 1-min stimulation-free intervals after the 5-min periods of stimulation and sham stimulation. During quiet wakefulness (Exp. 1, n = 16) and attentive wakefulness (supplementary experiment, n = 13), stimulation enhanced EEG power in the slow oscillation band (0.4–1.2 Hz) at prefrontal electrode sites, i.e., averaged across F7, Fz, and F8 (Upper), but not at centro-parietal sites, i.e., across C3, Cz, C4, P3, Pz, and P4 (stimulation \times lead: F_{1,120} = 2.44, P < 0.05, and F_{1,150} = 4.03, P < 0.01, respectively) (Lower). EEG power in the theta band (4–8 Hz) was enhanced over all recording sites (stimulation: A, F_{1,12} = 15.44, P < 0.005 and B, F_{1,15} = 12.85, P < 0.005, respectively). Stimulation during quiet wakefulness was additionally associated with a widespread change in beta band activity (15–25 Hz; stimulation: B, F_{1,15} = 21.47, P < 0.003). (Insets) Magnified changes in the 0.5–3 Hz range. Hatched areas indicate frequency bands significantly (P < 0.05) modified by tSOS.
Increased EEG Theta Activity and Improved Encoding. Transcranial SOS during waking enhanced encoding rather than consolidation of declarative memories, and this effect was associated with a widespread increase in EEG theta activity, but only a restricted increase in slow oscillation activity. Why was specifically theta oscillation enhanced by tSOS and not, for example, alpha activity dominant during quiet wakefulness (33)? One simple reason may be that during quiet wakefulness, alpha activity has a posterior focus that was less affected by tSOS location over the dorsolateral prefrontal cortex than EEG theta activity for which a prefrontal current source has been estimated (34). A preferred coupling between slowly oscillating potential fields ~0.75 Hz and oscillations at theta frequency may, however, reflect a basic functional relationship between the cortical networks underlying these oscillatory frequencies. Spontaneous slow oscillatory EEG activity emerges on a large scale at the transition into SWS, whereby theta activity prevails in the active waking state. Based on the correlation between EEG theta activity in waking and slow wave activity in sleep, it has been proposed that both oscillations are markers of a common homeostatic sleep process (35, 36). Neocortical slow oscillatory and hippocampal theta activity are also proposed to have complementary roles in the coupling of neocortical and hippocampal systems during hippocampus-dependent memory formation: Whereas theta is functionally related to working memory processes that enable the explicit encoding of episodic memories during active wakefulness, slow oscillations during SWS reveal a distinct grouping influence on hippocampal sharp-wave ripples and an associated neuronal replay of memory representations, which is thought to promote the redistribution of these representations to neocortical networks for long-term storage (9, 37–40). The generation of neocortical EEG slow oscillations depends at least partially on prior use of the same networks during encoding (11, 12, 41). Thus, in prefrontal-hippocampal networks involved in the formation of hippocampus-dependent memories, theta activity critical to explicit encoding in the waking brain may translate into slow oscillation activity during SWS, supporting the consolidation of these memories. However, the cellular mechanisms involved in the induction by tSOS of enhanced theta and, to a lesser extent, beta frequency activity and the wide-spread nature of these responses are presently unclear. The dependence of the theta enhancement by tSOS on the waking as opposed to the sleeping state points toward the relevance of a general increase in network excitability conveyed, among others, by increased cholinergic tone (42, 43). Increased network excitability might have been additionally promoted by the anodal component of tSOS.

A large body of data on performance-related EEG (and MEG) theta activity in rodents and humans has indicated its involvement in various aspects of memory processing, as well as in working memory, attention, and motivation-related functions (44, 45). In humans, in relation to episodic memory, enhanced EEG theta power and/or coherence have mostly been reported during encoding, but also during retrieval of verbal and spatial memories (3, 6, 7, 15, 46–48). During spatial navigation, theta activity increased with maze length, whereas at difficult maze junction, gamma rather than theta band activity was increased (48, 49), suggesting a permissive attention-related role for theta activity, whereas gamma activity (which for technical reasons was not measured here) more directly relates to the encoding and retrieval of specific information. On this background, the improved encoding performance observed here during tSOS is well in line with reported EEG theta enhancement in association with increased sustained attentional processes and working memory demands, although a more immediate contribution to the encoding process cannot be ruled out. A closer relation of theta activity to attentional aspects of stimulus processing might also explain why the overall moderate size of the tSOS effect on encoding became more robust for the last repetitions of the word list. The effect size might furthermore be related to the fact that our subjects were healthy university students showing optimum encoding capabilities under sham conditions.

Of note, the encoding improvement by stimulation was observed primarily for free recall measures (of the VLMT), but not for recognition measures (number recognition task). Unlike recognition, free recall requires the subject to generate his own retrieval cues and, hence, profits particularly from a high level of integration of an acquired memory representations into preexisting knowledge networks (24). Thus, tSOS enhancing later free recall, but not recognition may improve encoding efficiency primarily by increasing associative connectivity of acquired representations rather than by increasing the strength of associations per se. This view is also consistent with the concept of EEG theta oscillations during waking reflecting long-range cortical network activity (49). Increased associative connectivity as measured by long-range theta coherence during working memory and encoding tasks has been reported between prefrontal and temporoparietal neocortical regions (1, 4, 34, 48), and is interpreted as a functional binding of widely distributed cortical assemblies (49–51). Enhanced associative connectivity has likewise been observed between hippocampus and occipitotemporal neocortex using the same encoding paradigm as used in the present study (52). However, it has to be emphasized that these previous studies were all correlative in nature, not excluding that theta activity represents a mere epiphenomenon of task performance. By contrast, here, EEG theta activity was increased by electrical stimulation independent of the subject’s engagement in any task performance, providing evidence in humans for a functional significance of the EEG theta rhythm in facilitating processes of encoding (53). On this background, the finding of enhanced verbal encoding after tSOS provides strong evidence for a functional role of theta oscillations in the encoding of hippocampus-dependent memories.

In conclusion, the present study contributes essentially to the debate on the functional relevance of EEG oscillatory activity (54). Our findings support the concept that neocortical EEG theta activity is not merely an epiphenomenon of activity subserving sustained attention and encoding, but that cortical networks oscillating in theta frequency range are functionally relevant. Also, a physiological link between potential fields and networks oscillating at slow oscillation and theta frequencies is implicated.

Methods

Subjects and General Procedure. Twenty-eight subjects (19 to 35 years), all native German speakers, nonsmokers, medication-free, and of comparable education level (students or highly educated professionals) participated in two main experiments after having given informed written consent. Presence or history of epilepsy, paroxysms, cognitive impairments, mental, hormonal, metabolic, circulatory disorders, or sleep disturbances formed the exclusion criteria. The experimental protocol was approved by the ethics committee of the University of Lübeck.

For all experiments, subjects were first introduced to the experimental setup. They were asked to sit relaxed in a chair in a sound-attenuated room with dimmed light and a PC monitor. The experiments proper consisted of a stimulation condition and a sham stimulation condition, separated by an interval of at least 10 days and balanced in order across subjects. Sixteen subjects (nine female, seven male), mean age 23.8 ± 4.3 years (range: 19.4–34.7 years) participated in Exp. 1, and 12 subjects (nine female, three male),...
mean age of 23.8 ± 2.2 years (range 20.6–27.5 years) in Exp. 2. Thirteen subjects (six female, seven male; mean age of 24.08 ± 2.69 years; range 19.6–27.2 years) participated in a supplementary experiment.

Procedure of Experiment 1. Subjects arrived at the laboratory at 8:00 h. After preparation for tSOS and EEG recordings, subjects performed on tasks of declarative memory (verbal and nonverbal paired-associate learning) and procedural memory (auditory speech and mirror tracing), as illustrated in Fig. 1 (learning period). For details, see SI Methods. The order of tasks was balanced across subjects, with two different versions of each task also balanced across stimulation and sham conditions. Subjects were then seated in the recording room for continuous EEG recordings and tSOS. Stimulation started 20 min after the end of the learning period, with this postlearning delay approximately equivalent to that of a previous study with tSOS during postlearning sleep (18). EEG was recorded continuously between 20 min before stimulation and stimulation until 1 h after stimulation had ended while subjects watched dynamically shifting abstract color images on a PC monitor and listened to relaxing instrumental music. EEG recordings lasted until shortly before 12:00 h. Retrieval performance on the memory tasks was tested 7 h after the learning period, i.e., at 17:00 h. During the last 5 h of the 7-h retention period, participants could choose to engage in different standardized activities involving low cognitive demand and/or physical activity (e.g., taking a walk, bicycle riding). They were not allowed to take in coffee, tea, chocolate, or any kind of drugs, to read, watch or listen to music, or to stay awake. Also, they were asked not to rehearse any of the learned material. Adherence to these instructions was confirmed in a postexperimental interview (Fig. S1A).

To control for possible confounding influences of changes in arousal, mood, and motivation, before the learning and retrieval periods, the PANAS (55), and an adjective check list describing the subjects’ current mood and motivation on various dimensions (56) were applied (psychometric control tests; Fig. S1A). At retrieval, testing subjects performed on a word fluency task to assess the general capability to retrieve information from long-term memory (57), and on the digit span test of the Wechsler Adult Intelligence Scale to control for global changes in working memory function. Activation level was assessed by the Stanford Sleepiness Scale.

EEG Recordings and Analyses in Experiment 1. The EEG was recorded using Ag/AgCl electrodes placed according to the 10–20 system (at AFz, F7, F8, T3, T4, C3, C4, T4, P3, Pz, and P4), all referenced to an electrode attached to the nose. The ground was placed on the forehead. Recordings were amplified (1,000 µV/V), filtered between 0.05 and 30 Hz (5083 Syn-Amps; Neuroscan), and sampled with 200 Hz (5 ms) and 16-bit precision. Also, horizontal and vertical eye movements and the electromyogram (from the chin) were recorded for artifact detection. All recordings were stored on a PC for later offline analyses.

Analyses were conducted using Brain Vision Analyzer (Version 1.05; Brain Products). After applying an ocular correction to the entire EEG raw data signal (58), eight EEG intervals were selected for further EEG analyses: a 1-min interval before tSOS, five 1-min stimulation-free intervals immediately after each 5-min period of stimulation (including the interval after the last stimulation), and 1-min EEG epochs 30 and 60 min after the end of the fifth stimulation. EEG epochs were baseline corrected (0–10 s before stimulation and 10–20 s after stimulation), but were not taken into account if subjects were asleep. Performance data were not included in the analyses. Spaces were not filled by eye movements, blinks, or muscle artifacts. The EEG was re-referenced to the linked-earlobes in the following six frequency bands were calculated for all eight time intervals: slow oscillations (0.4–1.2 Hz), delta (1–4 Hz), theta (4–8 Hz), slow alpha (8–12 Hz), fast alpha (12–15 Hz), and beta (15–25 Hz).

Procedure of Experiment 2. Here, tSOS was applied during learning, i.e., while subjects encoded declarative memories. Subjects arrived at the laboratory at 9:00 h. After preparation for tSOS, they performed a modified version of the VLMT. Before and after the tSOS, subjects conducted a number list learning task. Immediately after the second number list learning task, the digit span test was applied to control for working memory function. Before and after the experiments, the subjects’ arousal level, mood, and motivation were assessed by PANAS, an adjective check list, and the Stanford Sleepiness Scale as described in the procedures of Exp. 1. The VLMT and number list learning were performed during the 5-min intervals of stimulation. Parallel versions of the tasks were balanced across the stimulation and sham conditions. In these experiments EEG was not recorded (Fig. S1B).

Learning Tasks in Experiment 2. In the VLMT (i.e., the German version of the Rey Auditory Verbal Learning Test), a standard word list consisting of 15 semantically unrelated words (nouns) was orally presented five times, with each run followed immediately by a free recall, in which the subject had to orally recall as many of the presented words as possible (R1–R5). Words from all lists were presented each for 1 s by a prerecorded neutral male voice. Subjects had unlimited time to recall the words. Immediately after the fifth run, a second list of 15 semantically unrelated words different from those in the standard word list (i.e., the IL) was presented in the same way as the standard list, and again, the subject had to recall as many of the words as possible. After the IL, subjects were requested to recall again the 15 words from the standard list, now without prior presentation of the list words (R6 in Fig. 2). Performance measures were the number of recalled words and the number of errors (defined as words named by the subjects but not contained in the corresponding list).

The number list learning task was adapted from ref. 59. The digit span test was used.

Statistical Analyses. Data were analyzed using SPSS version 11.5 for Windows. Normal distribution of data were assured by Kolmogorov-Smirnov test. EEG power was separately analyzed in the six frequency bands of interest using three-way analyses of variance (ANOVA) with the factors stimulation (stimulation vs. sham), time (baseline, 1-min stimulation-free intervals 1–5, 30 and 60 min after stimulation), and lead (F7, Fz, F8, T3, C3, Cz, T4, Pz, and P4). Three-way ANOVAs were also conducted using the difference in EEG power at the 7 time points referenced to the 1-min prestimulation baseline interval. Only robust results showing significance in analyses of both absolute and difference power values (and surviving Bonferroni correction) are reported here (referring to the analyses of absolute values). Post hoc t-tests were used to specify significant interactions.

Memory retention (defined by the difference in performance at retrieval testing and during learning) was analyzed by one-way ANOVA with the factors stimulation (stimulation vs. sham). One-way ANOVAs and Friedman ANOVAs were used separately for performance at learning and retrieval testing. Numbers of recalled words on the VLMT were first analyzed by two-way ANOVA with the factors stimulation (stimulation vs. sham) and 1-min stimulation-free intervals. Post hoc t-tests were used to specify significant interactions in these analyses.

ACKNOWLEDGMENTS. We thank Dr. Matthias Mölle for discussion of the manuscript, Prof. Vasil Koles for help with data analyses, and Sophie Peron, Max Rohwer, and Teodora Lambrinova for technical assistance. This work was supported by Deutsche Forschungsgemeinschaft Grant SFB 654.


Supporting Information

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SI Methods

Memory Tasks in Exp. 1. Four memory tasks were used, and for all tasks, parallel versions (A, B) were used in the subject’s two experimental sessions. To assess declarative memory, a word paired-associate learning task was used (1, 2). Forty-six semantically related pairs of German nouns (e.g., bird-claw) were sequentially presented on a monitor with a rate of 1/5 s and an interstimulus interval of 100 ms. Also, four dummy pairs of words at the beginning and end of each list served to buffer primacy and recency effects, respectively. At learning, before the retention period, presentation of the list was immediately followed by a cued recall, i.e., the subject was to respond by naming the second word on presentation of the first (cue) word of each pair, whereby the 46 stimulus words of the word list appeared on the screen in a different order than during the foregoing presentation. The subject had unlimited time to recall the appropriate response word. If a minimum of 60% correct responses was not obtained on a run, word-pairs were presented again in a newly randomized order (to prevent serial learning) and cued recall was repeated. At retrieval testing (17:00 h) in the afternoon cue words were again displayed in a newly randomized order, and the subject was required to recall the appropriate response words. Retention was measured by the difference in the number of words recalled at retrieval minus the number of words reproduced correctly at the last run of the learning period.

On a second declarative nonverbal paired-associate task, subjects were shown on the monitor a list of 16 evenly balanced pairs of either geometric or nongeometric line-drawings, adapted from ref. 3. During learning, presentation of the list was followed by a cued recognition task, in which subjects had unlimited time to recall the appropriate response drawing from a group of seven simultaneously presented other drawings. Learning ended when a minimum of 10 correct responses (60%) was reached on a run. At retrieval testing the cued recognition task was repeated using a newly randomized order of presentations. Retention was measured by the difference in the number of correctly retrieved drawings at retrieval testing minus the number of drawings correctly recognized at the last run of the learning period.

To test procedural memory, a finger sequence tapping task was used, adapted from ref. 4. Subjects were required to repeatedly finger-tap with the nondominant left hand a five-element sequence presented on a computer monitor as fast and accurately as possible on a key board. The two sequences used were “4-2-3-1-4” and “4-1-3-2-4.” The training period before sleep consisted of 12 30-s intervals with 30-s breaks between trials. Retrieval testing consisted of a practice run followed by three 30-s test intervals. A working memory component of the task was excluded by continuous presentation of the sequence on the screen. No feedback was given on pressing keys. Each 30-s interval was scored for the number of correctly completed sequences and the number of errors made. Performance at learning and retrieval testing was defined by averaged scores from the final three intervals during the learning period, and from three intervals of the retrieval period, respectively. Retention performance was defined by the difference between performance at retrieval testing minus performance during the learning period.

Also, procedural memory was evaluated by the mirror tracing task, adapted from refs. 2 and 5. In this task, subjects had to trace as fast and as accurately as possible line-drawn meaningless figures while these figures (with 26 to 27 angles and curved corners) and their hand movements were visible only through a mirror. Subjects traced each figure with an electronic stylus starting and ending at the same point. Drawing speed and error rate were registered. An error consisted of moving the stylus off the line of the figure. At learning, subjects first performed practice runs with a star-like figure until draw time was <1 min and <12 errors were made (the learning criteria), and then continued with 4 runs with the test figure. At retrieval testing, after one practice run, performance on 4 runs on the test figure was examined. On each occasion, the total time to trace a figure, and the number of errors were measured and averaged across the 4 runs. Retention was defined by the difference in performance on the test figure at retrieval testing and during the learning period.

The word fluency task conducted at retrieval testing to assess the general capability to retrieve information from long-term memory (6) requires the subject to write down within 2-min periods, respectively, as many kinds of either jobs or hobbies, and words starting with either the letter “M” or “P.” For the digit span test of the Wechsler Adult Intelligence Scale (7) subjects were to repeat accurately lists of orally presented digits forward and backward.

Transcranial Slow Oscillation Stimulation (tSOS). tSOS was applied for five 5-min epochs separated by 1-min stimulation free intervals. Stimulation parameters were the same as in a previous study of our lab (1, 8) and as follows. Size of stimulation electrodes, 0.502 cm²; current strength, 260 μV; current density, 0.517 mA/cm². The overall duration of 25-min of stimulation had been selected in our previous study as this duration is approximately equivalent to the length of the first slow wave sleep (SWS) epoch during nocturnal sleep. Stimulation was interrupted by 1-min stimulation-free intervals to enable the assessment of immediate effects of stimulation, because an uncontaminated EEG cannot be recorded during ongoing stimulation. Although EEG activity during these intervals cannot a priori be taken to reflect ongoing EEG activity during the 5-min stimulation epoch, there is strong evidence that activity immediately after stimulation with weak electric fields indeed reflects neuronal activity that has become entrained to the stimulation (9–12).

Results

Transcranial SOS During Quiet Wake Does Not Consolidate Memories. Changes in Performance Across the 7-h Retention Interval. Mean (±SEM) performance at learning and retention performance on the four different memory tasks in the stimulation and sham condition are summarized in Table 1 of the main text. Independent of the tSOS condition, performance on the declarative word paired-associate task remained unchanged (time: F1,15 = 0.42, P > 0.5) and decreased on the nonverbal paired-associate task (time: F1,15 = 6.43, P < 0.03) across the retention interval. Performance speed on both procedural finger sequence tapping (time: F1,15 = 23.46, P < 0.001) and the mirror tracing (time: F1,15 = 60.28, P < 0.001) increased across the wake retention period. Error rate on the finger sequence tapping task did not change across time (F1,15 = 1.31, P > 0.2), but decreased in the mirror tracing task (F1,15 = 8.97, P < 0.05), altogether indicating that improvements on these procedural tasks was not merely due to speed-accuracy trade-offs.
Improved Retention of Verbal Memory by tSOS Applied During Learning (Exp. 2). We additionally examined whether the effect of tSOS applied during encoding would manifest itself also at a delayed recall test 30 min later. Number of words recalled at this delay after testing on word list R6 was indeed increased after tSOS as compared with sham stimulation (sham: 13.08 ± 0.43, tSOS: 13.92 ± 0.42, P < 0.05). There was no significant difference in the number of errors (sham: 0.75 ± 0.28, tSOS: 0.33 ± 0.14, P > 0.1).

Fig. S1. Experimental procedures of Exp. 1 and 2. (A) In Exp. 1, subjects \( n = 16 \) participated in two sessions (tSOS and sham condition), with each consisting of a learning period, a 7-hour retention interval and subsequent retrieval testing. In the learning period, subjects acquired declarative (word paired-associates, nonverbal paired-associates) and procedural memories (finger tapping, mirror tracing). “Control tests 1” to assess mood, motivation and activation level (Positive and Negative Affect Schedule (PANAS), adjective check list) were conducted before learning and retrieval testing. Subjects spent the first 2 h of the retention interval in the laboratory sitting quietly and listening to relaxing music while the EEG was recorded continuously. Stimulation or sham stimulation was applied 20 min after the learning period. Stimulation consisted of five 5-min intervals of transcranial slowly oscillating electrical stimulation (S1-S5) each followed by a 1-min stimulation-free interval (white areas) used for EEG analyses. A 1-min interval immediately preceding S1 served as baseline. EEG analyses were also performed for 1-min intervals after stimulation by 30 and 60 min. At 12:00 h, subjects were released from the laboratory and engaged in standardized activities of low cognitive and physical demand. During the retrieval period, performance on the declarative and procedural tasks was retested. Subsequent “Control tests 2” assessed working memory and retrieval function per se (digit span, word fluency). (B) In Exp. 2, subjects \( n = 12 \) participated in two sessions (tSOS and sham condition). After assessment of self-reported tiredness, mood and motivation in Control tests (Stanford Sleepiness Scale, PANAS, adjective check list) subjects performed on two learning tasks, the number list learning task (NL), and the adapted Verbal Learning and Memory Test (VLMT) while either tSOS or sham stimulation was applied. As in Exp. 1, stimulation consisted of five 5-min intervals (S1-S5) each followed by 1-min stimulation-free intervals (white area). The period of stimulation and the learning tasks began simultaneously after a 2-min period of quiet wakefulness. Learning of the tasks took place essentially only during acute tSOS intervals. The number list learning task was conducted twice (no. 1, no. 2) with two different lists. The VLMT consisted of 5 presentations of the standard word list each immediately followed by a free recall (R1-R5), then an interference list (IL) of words was presented, which was to be immediately recalled before free recall of the standard word list was tested again (R6). Working memory performance on the digit span test (Digits) was tested immediately after the 5th interval of stimulation.
Fig. S2. Changes over time in EEG spectral power in delta, alpha, and beta bands of Exp. 1. EEG spectral power across the five 1-min intervals immediately succeeding the stimulation intervals and 30 and 60 min after termination of stimulation in the delta (1–4 Hz), slow alpha (8–12 Hz), fast alpha (12–15 Hz), and beta bands (15–25 Hz) averaged across all locations. **, P < 0.01; *, P < 0.05 (t test) for comparisons between the stimulation and sham condition (n = 16). Note, during some intervals, strong modifications in the theta frequency band affected adjacent activity in the delta band, resulting in increased activity as compared with sham stimulation for the 2nd and 4th stimulation-free intervals (stimulation × time: F_{7,105} = 3.85, P < 0.05).
Table S1. Performance on the psychometric control tests in experiment 1 (n = 16; mean ± SEM)

<table>
<thead>
<tr>
<th>Test</th>
<th>Sham</th>
<th>Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before learning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANAS, positive score</td>
<td>5.11 ± 0.52</td>
<td>5.23 ± 0.59</td>
</tr>
<tr>
<td>PANAS, negative score</td>
<td>3.70 ± 0.41</td>
<td>3.74 ± 0.37</td>
</tr>
<tr>
<td>After retrieval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit span forward (digits)</td>
<td>8.18 ± 0.21</td>
<td>8.38 ± 0.22</td>
</tr>
<tr>
<td>Digit span backward (digits)</td>
<td>6.62 ± 0.32</td>
<td>6.94 ± 0.30</td>
</tr>
<tr>
<td>Word fluency (words)</td>
<td>22.50 ± 1.30</td>
<td>25.25 ± 1.41</td>
</tr>
<tr>
<td>Stanford Sleepiness Scale</td>
<td>2.37 ± 0.36</td>
<td>2.50 ± 0.35</td>
</tr>
<tr>
<td>PANAS, positive score</td>
<td>4.88 ± 0.47</td>
<td>5.14 ± 0.51</td>
</tr>
<tr>
<td>PANAS, negative score</td>
<td>3.48 ± 0.36</td>
<td>3.73 ± 0.38</td>
</tr>
</tbody>
</table>

For all measures, sham vs. stimulation comparisons were nonsignificant.
Table S2. Error subtypes on the VLMT in Exp. 2 (n = 12; mean ± SEM)

<table>
<thead>
<tr>
<th>Type of error</th>
<th>Sham</th>
<th>Stimulation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>False memories (word not in the standard list)</td>
<td>0.17 ± 0.09</td>
<td>0.21 ± 0.13</td>
<td>0.81</td>
</tr>
<tr>
<td>Perseverations (repetitions)</td>
<td>0.46 ± 0.19</td>
<td>0.16 ± 0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>Accuracy (ratio of total correct words by total cited words)</td>
<td>0.96 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.35</td>
</tr>
</tbody>
</table>

For all measures, sham vs. stimulation comparisons were nonsignificant.