Sleep-Dependent Improvement in Visuomotor Learning: A Causal Role for Slow Waves

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Study Objectives: Sleep after learning often benefits memory consolidation, but the underlying mechanisms remain unclear. In previous studies, we found that learning a visuomotor task is followed by an increase in sleep slow wave activity (SWA, the electroencephalographic [EEG] power density between 0.5 and 4.5 Hz during non-rapid eye movement sleep) over the right parietal cortex. The SWA increase correlates with the postsleep improvement in visuomotor performance, suggesting that SWA may be causally responsible for the consolidation of visuomotor learning. Here, we tested this hypothesis by studying the effects of slow wave deprivation (SWD).

Design: After learning the task, subjects went to sleep, and acoustic stimuli were timed either to suppress slow waves (SWD) or to interfere as little as possible with spontaneous slow waves (control acoustic stimulation, CAS).

Setting: Sound-attenuated research room.

Participants: Healthy subjects (mean age 24.6 ± 1.0 years; n = 9 for EEG analysis, n = 12 for behavior analysis; 3 women)

Measurements and Results: Sleep time and efficiency were not affected, whereas SWA and the number of slow waves decreased in SWD relative to CAS. Relative to the night before, visuomotor performance significantly improved in the CAS condition (+5.93% ± 0.88%) but not in the SWD condition (-0.77% ± 1.16%), and the direct CAS vs SWD comparison showed a significant difference (P = 0.0007, n = 12, paired *t* test). Changes in visuomotor performance after SWD were correlated with SWA changes over right parietal cortex but not with the number of arousals identified using clinically established criteria, nor with any sign of "EEG lightening" identified using a novel automatic method based on event-related spectral perturbation analysis.

Conclusion: These results support a causal role for sleep slow waves in sleep-dependent improvement of visuomotor performance.

Keywords: Arousals, slow wave activity, sleep homeostasis, slow wave deprivation, visual motor learning

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A NUMBER OF STUDIES HAVE SHOWN THAT SLEEP CAN ENHANCE PERFORMANCE OF PREVIOUSLY LEARNED TASKS.[1] SOME STUDIES SUGGEST THAT A COMBINATION of both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep is important,[2,3] whereas others emphasize either NREM or REM sleep.[4] However, assigning a role in memory enhancement to different stages of sleep, or to a combination of stages, is problematic because sleep function likely depends on specific electrophysiologic events, such as slow waves or spindles, rather than on conventionally defined sleep stages. Furthermore, most experiments so far have been correlative. Thus, it is important to determine whether sleep-dependent improvements in performance are causally related to specific electrophysiologic features of sleep.

During NREM sleep, virtually all cortical neurons undergo approximately 1-Hz slow oscillations, characterized by periods of membrane depolarization and firing activity (up states) followed by periods of membrane hyperpolarization and cessation of firing (down states).[5] Slow oscillations are synchronized by cortico-cortical connections,[6,7] giving rise to slow waves of low frequency (0.5-4.5 Hz) and high amplitude (often > 75 uV) that can be recorded in the sleep electroencephalogram (EEG). This slow wave activity (SWA, the EEG power density between 0.5 and 4.5 Hz during NREM sleep) is thought to be a marker of sleep need, in that it is high at sleep onset, declines during sleep, is further increased after sleep deprivation, and is reduced by naps.[8-10]

Recently, we used a visuomotor adaptation task in which subjects learn to reach for targets on a visual display while the cursor trajectory, unbeknownst to them, is rotated by the computer.[11] We showed that visuomotor performance in this task was enhanced by a night of sleep but not by an equivalent period of wakefulness and found, using high-density EEG, that learning led to a local increase in sleep SWA over a right parietal cortical area involved in rotation adaptation. Moreover, we found that the postsleep improvement correlated with the local increase in sleep SWA.[11] These results demonstrated that sleep slow waves are regulated locally as a consequence of learning and raised the possibility that they may be causally involved in sleep-dependent visuomotor performance improvements. To test this hypothesis in the current study, subjects learned the same rotation task,[11] after which sleep slow waves were disrupted with acoustic stimuli. We investigated whether selective slow wave deprivation (SWD) prevented the improvement in postsleep visuomotor performance and whether changes in visuomotor performance correlated with changes in SWA.

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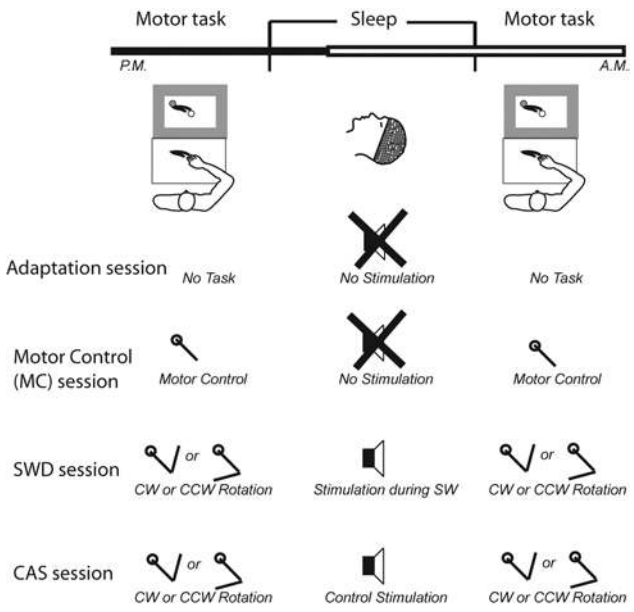

Figure 1—Study Design. The study consisted of 4 conditions, spaced ~ 1 week apart and in randomized order. The motor control (MC) task involves the same number of movements as the rotation adaptation task, but no rotation adaptation occurs. SWD refers to slow wave deprivation; CAS control acoustic stimulation; CW, clockwise; CCW, counterclockwise.

METHODS

Experimental Design

Twelve healthy, medication free, right-handed subjects (3 women; mean age 24.6 ± 1.0 years) participated in the study, but the final EEG analysis was based on 9 subjects because 2 subjects failed to complete all 4 conditions, and the EEG data for 1 subject in 1 condition were lost due to technical problems. Prior to the study, subjects filled out a written questionnaire in which they reported no history of excessive daytime sleepiness, sleep complaints, or primary sleep disorders (including obstructive sleep apnea and periodic limb movements of sleep), with a normal bedtime of 23:45 ± 19 minutes and wake time of 07:13 ± 14 minutes. The study was approved by the Institutional Review Board of the University of Wisconsin-Madison, and all subjects signed an Institutional Review Board-approved informed consent form.

The study consisted of 4 overnight conditions: an adaptation condition, a motor control (MC) condition, a SWD condition, and a control acoustic stimulation (CAS) condition (Figure 1). Following the adaptation night, the order of MC, SWD, and CAS was randomized across subjects, with the conditions spaced at least 1 week apart. One week prior to, and for the entire duration of, the study subjects were instructed to refrain from alcohol, caffeine, and nicotine and to maintain regular sleep-wake schedules. Compliance was verified with self-reported sleep logs and wrist motor actigraphy (Actiwatch, Mini-Mitter, Inc., Bend, OR).

Subjects arrived in the laboratory between 20:00 and 21:00, were outfitted with high-density EEG (256 electrode nets, Electrical Geodesics, Eugene, OR), performed a motor task just before going to bed (MC, SWD, or CAS only), and then slept in the laboratory (lights out: 23:27 ± 9 min, mean ± SEM). The next morning, after being woken up at their normal waking time, subjects were retested on the motor task.

Motor Tasks

Two previously well-characterized tasks[12-15] were used. The motor control task was performed in the MC condition only: subjects used their right arm to move a cursor on a digitizing tablet with out-and-back movements, starting from a central position and aiming at 1 of 8 targets displayed on a computer screen together with the cursor position. Targets were presented at the rate of 1 per second, and subjects were instructed to move as soon and as fast as possible, without correcting their movements. Subjects performed 20 blocks of 90 movements each. The other task, which involves a visuomotor adaptation to a rotated display, was performed in both the SWD and CAS condition. This task has similar kinematic requirements and is subjectively indistinguishable from the control task; positron emission tomographic studies have shown that it specifically activates the right posterior parietal cortex,[12] whereas EEG studies have found that learning this task is followed, in the same cortical region, by a local increase in sleep SWA.[11] In the CAS and SWD conditions, after a block of 90 movements without rotation, unbeknownst to the subject, the cursor position on the screen was rotated either clockwise (CW) or counterclockwise (CCW) relative to the hand position in 4 incremental steps of 15°, up to 60° (each step comprised 3 blocks of 90 movements each, for a total of 1080 movements). The degree of learning achieved at the end of the training session was assessed in a separate block (test block). The following morning visuomotor performance was tested in the same way (retest block) after a refresher block. Learning of CW rotation does not transfer to CCW rotation,[13] and, therefore, the same subject learned a CW and a CCW rotation in 2 separate conditions (SWD and CAS), allowing intrasubject comparisons.

Directional error at peak velocity, reaction time, movement time, peak velocity, and peak acceleration were computed for each movement. Directional error was measured as the difference in degrees between the direction of the vector from the start to the target and that of the vector from the start to the movement peak velocity.[12] Rotation adaptation indicates the extent to which directional error to an imposed rotation improves from the training session to the postsleep session. Rotation adaptation was computed using the directional error at the peak velocity in the test and retest blocks, expressed as 100 * (1-[mean directional error / 60°]).[14] Postsleep improvement was computed as the difference in mean directional error between the test and retest block.

Slow Wave Deprivation

The method for SWD is similar to that used in other studies,[16-19] and, as in 1 case before,[19] tones of various frequency and duration, rather than a single tone, were used. Moreover, prior to the SWD condition, for each subject, sleep slow waves from either the MC (if available) or adaptation night were counted and characterized (period, amplitude, etc.). Then, the subject's wave characteristics

were fed into a custom written, semiautomated, slow-wave detection program (Labview, National Instruments, Austin, TX). With the a-priori knowledge of a subject's slow-wave characteristics, this program allows the experimenter to continuously monitor the EEG tracings (using any number of channels) in real time and deliver a tone (500-2000 Hz; 40-110 DB; 1-5 s) through a loudspeaker whenever slow waves are detected (0.5-4.5 Hz; > 75 uV). For this study, a single frontal channel (Fz) was monitored, based on the previous demonstration with high-density EEG that the majority of slow waves originate in frontal regions.[6] If no response occurred, the experimenter increased the tone volume via a click-and-drag slider using a linear scale in the custom software program, a different tone frequency was played, or the tone duration was increased. A response was defined as any shift in sleep stage, the occurrence of a K-complex, the appearance of EEG activation (high-frequency, low-voltage EEG) or alpha activity, or an increase in muscle tone. In contrast with previous studies, in the CAS condition, a similar number of stimuli was administered during NREM sleep, but only when the EEG did not show slow waves. Just as for SWD, the goal for CAS was to produce a change in the sleep EEG (testifying that the stimulus had indeed affected ongoing brain activity) while, at the same time, avoiding a shift in sleep stage. Periods with little SWA constitute lighter sleep relative to periods with high SWA, as indicated by lower arousal thresholds.[20,21] Thus, the intensity of the acoustic stimuli during CAS had to be slightly lower (by 20% on a linear volume scale) than during SWD. When CAS preceded SWD, the experimenter delivered approximately 450 tones during the entire night, a number based upon previous experience. In the MC condition, subjects were allowed to sleep undisturbed.

Sleep Recordings

All-night EEG recordings were sampled at 500 Hz, downsampled to 128 Hz, band-passed (2-way least-squares FIR) filtered between 0.5 and 40 Hz, and average referenced. Submental electromyogram recordings were downsampled and band-passed filtered between 20 and 40 Hz, whereas electrooculogram (EOG) recordings were downsampled and band passed between 0.5 and 40 Hz. Sleep staging was based on EEG electrodes C3 and C4 referenced against mastoid electrodes A1 and A2, as defined by the international 10/20 system. Sleep stages were visually scored in 20-second epochs according to standard criteria.[22] All recordings were scored by 2 of the authors, one of whom is a board-certified sleep physician (RB). Both scorers were blinded to the experimental condition, and interscorer reliability was greater than 90% (using laboratory gold-standard recordings, interscorer correlation was also > 90%). Scoring of arousals was done according to established arousal criteria[23] by the authors blinded to experimental condition. To demonstrate scoring reliability, as done previously,[24] 4 recordings from the dataset were rescored by the same scorer. The arousal scoring reliability from these 4 recordings was 94% ± 2% (mean ± SEM).

As in previous studies,[11,25] EEG power spectral analysis was performed in consecutive 4-second epochs (fast Fourier transform routine, Hanning window) for all 256 channels. Artifacts were excluded by visual inspection and semiautomatically.[11] An automatic wave-detection procedure[26] was used for the quantitative analysis of slow waves. The event-related spectral perturbation (ERSP) analysis of the EEG response to a tone was conducted using the Matlab (MathWorks, Natick, MA) plug-in software EEGLAB.[27]

Sleepiness and Vigilance

Reaction time, movement time, and peak velocity of the motor task in the evening and the following morning blocks were measured in all subjects. In 5 subjects the Stanford Sleepiness Scale [28] and the Psychomotor Vigilance Task (total duration 10 minutes, interstimulus interval between 2 and 10 s)[29] were administered both before and after the motor task in the evening and the following morning.

Statistics

To assess changes in sleep parameters, we used paired *t* tests and compared data from the MC, SWD, and CAS conditions. To investigate changes in the EEG power density between the SWD and CAS conditions, the entire EEG power (1-25 Hz; 0.5-Hz frequency bins) was expressed as a percentage of the mean power density in the MC condition. Paired *t* tests were used to compare MC, SWD, and CAS conditions for either individual electrodes or the mean of all scalp electrodes for each frequency bin. To investigate changes in ERSP between conditions, a 2-tailed bootstrap analysis was performed.[30] As in previous studies,[11,25] statistical nonparametric mapping (SnPM; simple-threshold P = 0.05 permutation test controlling for multiple comparisons) was used to determine the effect of SWD on the SWA topographic pattern.[31] The effect of SWD on the homeostatic decline of SWA was assessed using a paired *t* test with Bonferroni corrections. Mixed-model analyses of variance (ANOVA) with posthoc tests (with correction for multiple comparisons) were used to ascertain changes between blocks and conditions for all behavior measures. In addition, behavior measures were correlated (Pearson) with EEG power density values in the SWD condition for individual electrodes and for the mean of all scalp electrodes for each frequency bin. To ascertain which sleep variables were responsible for behavior changes, we performed a multiple-regression analysis using a general linear model and report the adjusted R^2 (proportion of variance accounted for by the model) as well as, for each independent variable, the β value (regression coefficient expressed in units of standard deviations).

RESULTS

Effects of SWD on Sleep Time and Architecture

Each subject was recorded for 4 nights using high-density EEG (Figure 1). During the CAS condition, subjects received approximately the same number of acoustic stimuli as in the SWD condition but during periods of NREM sleep when there were no detectable slow waves in the EEG. The number of tones did not statistically differ between SWD and CAS (mean ± SEM, n = 9, SWD = 507 ± 95, CAS = 412 ± 63; P = 0.26, T = -1.195, paired *t* test).

Figure 2 shows a representative EEG trace when two, 1-second tones were played and the subject went into a lighter state

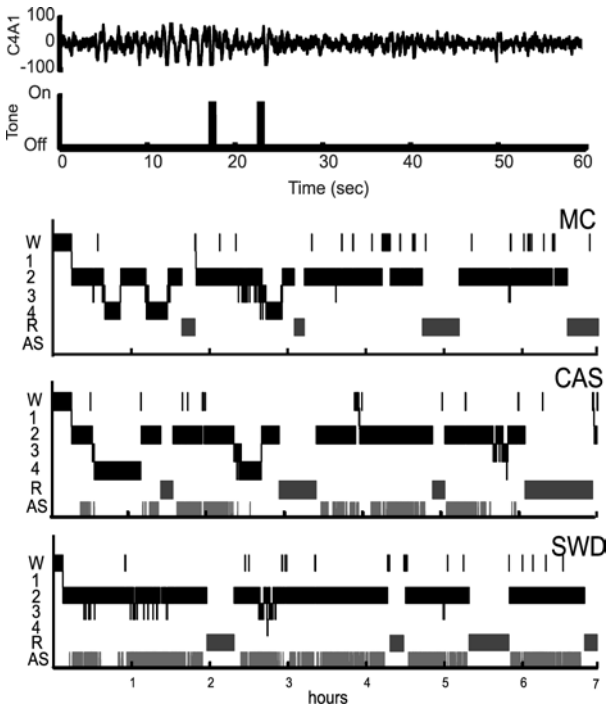

Figure 2—Representative electroencephalographic traces and hypnograms. Top trace shows when two, 1-second duration tones at ~ 85 dB caused the subject to transition into a lighter stage of sleep. Bottom shows representative hypnograms for motor control (MC), control acoustic stimulation (CAS), and slow wave deprivation (SWD) conditions; W, waking; R, rapid eye movement sleep; 1-4: non-rapid eye movement sleep stages 1-4; AS, time of acoustic stimulation.

of sleep, as well as representative hypnograms during the MC, SWD, and CAS conditions. Table 1 summarizes measures of sleep quality and quantity for the MC, SWD, and CAS conditions. Previous studies in humans had shown that, during SWD, relative to a night of undisturbed sleep, the duration of slow wave sleep (SWS) can be reduced by 40% to 90%.[16-19] In the current study, we obtained comparable results. Specifically, during SWD compared with MC, SWS was reduced by 57% and stage 2 was increased by 21%, whereas the duration of total sleep and REM sleep were similar (Table 1A). Wake time after sleep onset was also similar in the 2 conditions, but the number of arousals per hour (arousal index; as defined by[23]) increased in SWD relative to MC (Table 1A).

The comparison between SWD and CAS is the most informative because it is not confounded by possible aspecific effects of the acoustic stimulation. CAS did not significantly affect the duration of SWS, REM sleep, total sleep, or wake time after sleep onset (Table 1A). As intended, on the other hand, the duration of SWS was significantly reduced by 57% in SWD relative to CAS, whereas the duration of stage 2 increased by 19% (Table 1A). The percentage of stage 1 sleep was also higher (+57%) in SWD, compared with CAS. Moreover, although only a small minority (~6%) of all tones produced arousals as classically defined,[23] there were approximately 5 more arousals per hour in the SWD than in the CAS condition (arousal index, Table 1A). A retrospective analysis of the EEG traces indicated that the few occasions during SWD in which the auditory stimulus induced an arousal were due to the tone inadvertently (because of the on-line nature of the procedure) falling on a segment of lighter sleep (with no slow waves). Since, during the SWD condition, stimuli were 20% louder than in the CAS night (see Methods), they were presumably more likely to produce an arousal if administered incidentally when slow waves were not present.

SWD Globally Reduces SWA and Flattens its Homeostatic Decline Across the Night

Previous studies have shown an approximately 30% to 40% decrease in SWA during SWD.[16-19] Here, NREM SWA during SWD was reduced by 20% relative to MC and, more importantly, by 31% relative to CAS (Table 1B). During SWD, relative to both MC and CAS, the average EEG power across all 256 electrodes was significantly reduced between 1 and 6.25 Hz (paired *t* test, n = 9, P < 0.05; Figure 3, top panel), whereas there was no difference in the NREM EEG power spectrum between CAS and MC.

A recent study using automatic wave-detection algorithms at all EEG electrodes found that the decline of SWA in sleep is correlated with a decrease in the number of slow waves.[26] Consistent with these results, SWD decreased the number of NREM slow waves by 42% relative to MC and by 52% relative to CAS (Table 1B). Previous studies also reported correlations between sleep-dependent improvement in performance in some tasks[1] and spindle activity.[32-37] SWD caused a 13% increase in NREM spindle density relative to MC, but there was no difference relative to CAS (Table 1B).

We previously found that learning the visuomotor task is followed, during the first 30 minutes of NREM sleep, by an increase in sleep SWA at 6 EEG channels positioned over right parietal areas.[11] In this study, we therefore identified, within the right parietal region and for each subject, the electrode with the largest SWA increase in CAS relative to MC and then measured how SWD affected the NREM EEG power spectrum at this "peak" channel (Figure 3, bottom panel). The EEG power recorded from this channel did not differ between CAS and MC when computed across the entire night. During the first 30 minutes of NREM sleep, however, SWA in the peak channel was higher in CAS relative to MC, although the change did not reach significance (paired *t* test, n = 9, P = 0.13). By contrast, for the entire night, the NREM EEG power at the peak channel was significantly reduced between 0.5 and 6.25 Hz in SWD compared with MC (paired *t* test, n = 9, P < 0.05) and, more stringently, in the frequency ranges 0.25 to 3.75, 5.25, and 6.25 Hz in SWD compared with CAS (paired *t* test, n = 9, P < 0.05). Almost identical results were obtained when the EEG power was averaged across all 6 right parietal channels (data not shown).

SWA shows high frontal-region predominance with a wedge-shaped pattern,[38-40] and the individual's SWA pattern is highly reproducible across nights.[41,42] Figure 4 shows the topographic distribution of NREM SWA during the 3 experimental conditions. The expected frontal, wedged-shaped SWA pattern was present not only in MC and CAS, but also in SWD. However, relative to CAS, SWD showed a significant global decrease (range 15%-50%) in SWA that spanned frontal, parietal, and temporal regions

Table 1—Sleep Measures During the 3 Experimental Conditions

A	MC	SWD	CAS	SWD vs MC	CAS vs MC	SWD vs CAS
TST, min	397 ± 9.7	380 ± 9.8	391 ± 8.4	0.165	0.443	0.363
SE %	90.1 ± 1.3	88.8 ± 1.6	90.6 ± 1.0	0.462	0.716	0.462
WASO, min	31.3 ± 6.4	32.6 ± 5.9	24.7 ± 3.5	0.839	0.287	0.328
AI	7.2 ± 1.1	13.2 + 1.4	8.1 + 1.0	0.0033	0.54	0.0045
Sleep stage						
1, min	13.3 ± 2.0	27.1 ± 6.2	17.3 ± 2.9	0.063	0.247	0.062
1, %	3.5 ± 0.6	7.3 ± 1.7	4.5 ± 0.8	0.061	0.260	0.050
2, min	191.0 ± 13.0	230.9 ± 13.4	193.7 ± 7.6	0.014	0.800	0.026
2, %	47.9 ± 2.8	60.4 ± 2.4	49.5 ± 1.9	0.002	0.528	0.04
SWS, min	97.9 ± 8.9	41.5 ± 9.2	97.2 ± 7.7	0.0001	0.885	0.0001
SWS, %	24.5 ± 1.9	11.1 ± 2.6	24.8 ± 1.9	0.0002	0.828	0.0008
REM, min	94.9 ± 5.0	80.7 ± 6.3	83.5 ± 7.1	0.151	0.271	0.788
REM, %	24.1 ± 1.6	21.1 ± 1.2	21.2 ± 1.7	0.184	0.287	0.951
B	**MC**	**SWD**	**CAS**	**SWD vs MC**	**CAS vs MC**	**SWD vs CAS**
SWA, %	100	80.0 ± 6.8	111.6 ± 12.6	0.009	0.192	0.007
Slow waves[a]	1326.5 + 205.4	772.0 ± 102.2	1611.9 ± 142.5	0.019	0.053	0.0008
Spindle						
Power (12-15 Hz), %[a]	100	112.7 ± 7.1	103.3 ± 4.4	0.117	0.476	0.061
Density	0.68 ± 0.07	0.77 ± 0.08	0.74 ± 0.07	0.017	0.101	0.424
Amplitude μV	10.84 ± 1.02	11.30 ± 1.10	10.96 ± 0.92	0.077	0.627	0.286
Duration, s	1.19 ± 0.04	1.22 ± 0.04	1.20 ± 0.04	0.158	0.573	0.271

Data are expressed as mean ± SEM (n = 9; paired *t* test). **(A)** slow-wave sleep (SWS; non-rapid eye movement [NREM] sleep stages 3+4) expressed in minutes and as a percentage of total sleep time (TST). Arousal Index (AI) is the number of arousals per hour. **(B)** slow wave activity (SWA) and electroencephalographic power in the spindle range in slow wave deprivation (SWD) and control acoustic stimulation (CAS) are expressed as a percentage of the power in the same frequency range during motor control (MC), calculated from the mean of all channels. "Slow waves" indicates the number of slow waves during all NREM epochs. Spindle density is the number of spindles per minute calculated from the mean of all channels for all NREM epochs. SE refers to sleep efficiency; WASO, wake time after sleep onset.

[a]Data from 1 subject were removed from the analysis of slow waves and spindle power because values in the MC condition were > 3 SD from the mean.

Table 2—Results of Mixed-Model Analysis of Variance for Directional Error in Step-Wise Learning

	Step	Block	Condition	Step Block	Condition × Sleep	Block × Condition	Step × Block × Condition
df	3,260	2,260	1,260	6,260	3,260	2,260	6,260
f	37.56	25.15	0.02	0.18	0.06	0.13	0.07
P	< 0.0001	< 0.0001	> 0.8	> 0.9	> 0.9	> 0.8	> 0.9

(SnPM). By contrast, there was no change in SWA between CAS and MC (data not shown). Relative to both MC and CAS, SWD also showed a trend toward a global increase in spindle (12- to 15-Hz) power that was more pronounced (~40%) over frontal regions (Supplementary Figure 1; see *SLEEP* website at www.journalsleep.org), but no single channel showed statistically significant differences (SnPM).

Figure 5 shows the overnight time course of SWA for MC, CAS, and SWD. As expected, SWA showed the normal homeostatic decline during MC and CAS but remained at a constant low level during SWD. SWA during the first sleep cycle was significantly different between SWD and CAS (n = 9, paired *t* test, P < 0.0001 with Bonferroni correction).

SWD Prevents Next-Day Improvement in Visuomotor Performance

All subjects learned to adapt to the 2 rotations (CW and CCW), and the decrement of mean directional error across blocks was significant, with no differences between CAS and SWD (Figure 6A and Table 2). However, although at the end of the first 15° incremental step adaptation was virtually complete, the directional error at the end of the other steps was progressively higher than the corresponding baseline values. At the third block of the 60° step, there was a residual mean directional error of approximately 8° in both adaptation conditions, which was significantly greater than the baseline mean directional error (Figure 6A, $F_{1,42} = 35.28$ P < 0.0001, n = 12). On the other hand, reaction times as well as peak velocities at the end of the training were not different from baseline (Supplementary Table 1, all: P > 0.6, n = 12; see *SLEEP* website at www.journalsleep.org). After training in the evening, the degree of learning achieved was tested in a separate movement block. On average, subjects showed a rotation adaptation of 59% (corresponding to a mean directional error of 24°; a perfect rotation adaptation of 100% would correspond to a mean directional error of 0º) with no differences between CAS and SWD (Figure 6B, white

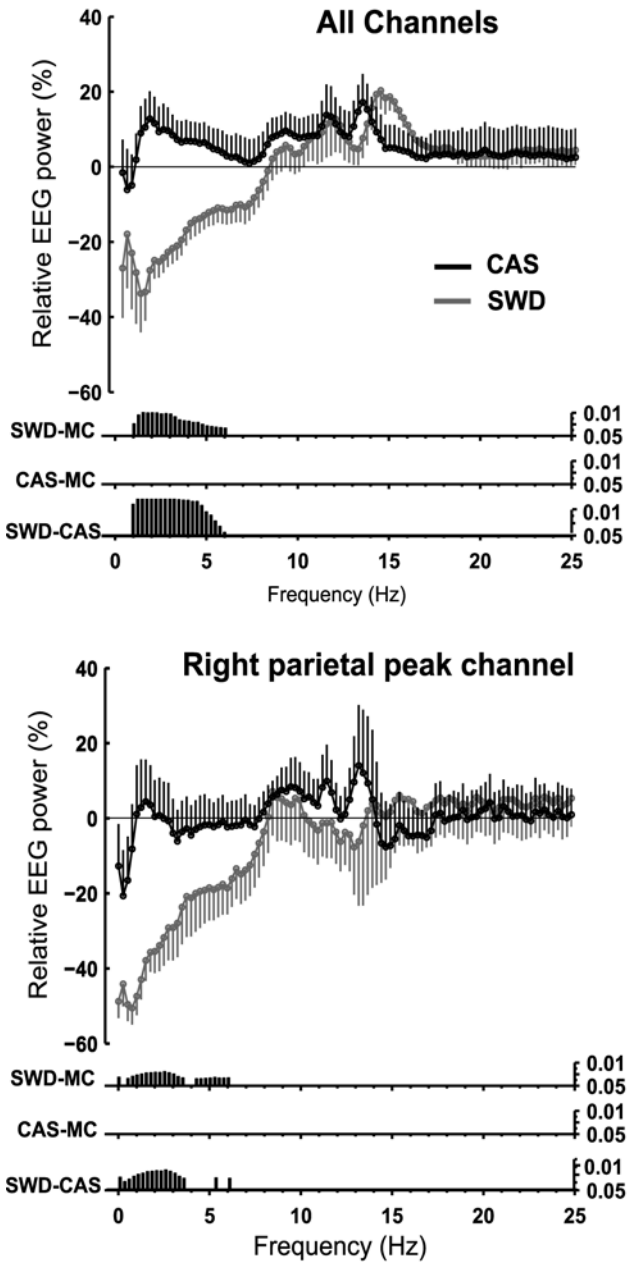

Figure 3—All-night changes in non-rapid eye movement (NREM) electroencephalographic (EEG) power spectrum for control acoustic stimulation (CAS) and slow wave deprivation (SWD) relative to motor control (MC) (0 line) for all channels (top) and for a single channel (bottom) over right parietal cortex. Black bars indicate frequency bins for which EEG power was significantly different between conditions (mean ± SEM, n = 9, paired *t* test).

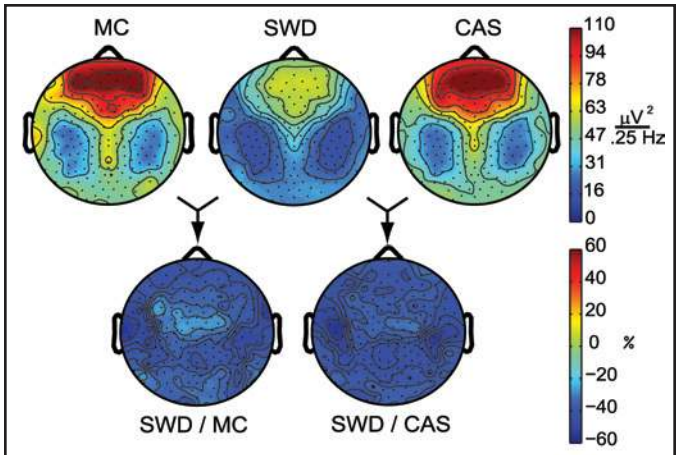

Figure 4—Topographic distribution of slow wave activity (SWA) during non-rapid eye movement (NREM) sleep in motor control (MC), slow wave deprivation (SWD), and control acoustic stimulation (CAS). Top, average NREM SWA for the entire night (n = 9). Color bar values represent the absolute electroencephalographic power (μV^2/0.25-Hz frequency bin) averaged for the 0.5-4.5 Hz range. Values were plotted at the corresponding position on the planar projection of the scalp surface, and interpolated (biharmonic spline) between electrodes (dots) using EEGLAB.[27] Bottom: Topographic distribution of the slow wave activity in the SWD condition, expressed as the percentage difference relative to MC or CAS for the entire night.

bars). These results are similar to those we have previously reported for different groups of subjects.[11] Adaptation rate was not affected by either rotation type or order of performance ($P > 0.6$). Thus, the degree of learning was the same in the CAS and SWD conditions before sleep. However, after sleep rotation adaptation improved in the CAS but not in the SWD condition ($P = 0.0007$, n = 12, paired *t* test; Figure 6B, black bars). All individual subjects, with 1 exception, performed better (reduced mean directional error) after sleep in the CAS than in the SWD condition (Figure 6C). Thus, an approximately 50% decrease in sleep slow waves and an approximately 30% decrease in SWA are sufficient to prevent the sleep-dependent improvement in visuomotor performance of the rotation-adaptation task.

Since sleepiness or changes in vigilance/attention can affect performance on motor tasks,[29] we analyzed reaction time, movement duration, and peak velocity in the motor task using mixed-model ANOVA and found no differences between evening and morning or between conditions (CAS or SWD; Supplementary Table 1A; see *SLEEP* website at www.journalsleep.org). To confirm these findings, subjective sleepiness was also measured in a subset of subjects (n = 5) using the Stanford Sleepiness Scale.[28] Subjective sleepiness decreased from evening to morning, but there was no difference between CAS and SWD (Supplementary Table 1B; see *SLEEP* website at www.journalsleep.org). In the same subset of subjects, vigilance—as measured by the Psychomotor Vigilance Task[29]—did not show any difference between morning and evening or between CAS and SWD (Supplementary Table 1B), although, as for the Stanford Sleepiness Scale results, the small sample size for the Psychomotor Vigilance Test data may have limited our ability to detect significant effects.

Experimentally Induced Changes in Sleep Slow Waves and Postsleep Visuomotor Performance Improvements are Correlated

Having established that the arousals were few, but significantly different between conditions, it became essential to ascertain whether the decrease in performance after SWD was due to the decrease in SWA and/or to the increase in arousal index. Figure 7A shows percentage changes in NREM sleep SWA plotted against postsleep changes in visuomotor performance in SWD and CAS. For every subject (with 1 exception), higher SWA values were associated with better performance,

and lower SWA values with worse performance. This relationship between SWA and visuomotor performance was present for both the SWA averaged across all channels (left panel) and the SWA of the peak channel over the right parietal cortex (right panel). Likewise, when there was a greater percentage change in the number of NREM slow waves (lower panels), there was a greater improvement in visuomotor performance (Figure 7B). Moreover, as shown in Figure 8A, the correlation between EEG power changes in the peak channel and visuomotor performance changes after SWD was specific for the low SWA frequency range (1.0-2.25 Hz, n = 9, $P < 0.05$). Finally, Figure 8B shows that the correlation between SWA and visuomotor-performance changes after SWD was topographically specific, being significant only over right parietal cortex. In contrast, there was no statistically significant correlation between the change in visuomotor performance for the SWD condition and the arousal index ($P = 0.06$, $r = -0.63$, $n = 9$), the number of tones delivered ($P = 0.44$, $r = -0.30$, $n = 9$), or the percentage of time spent in different sleep stages (eg, stage 1, $P = 0.61$, $r = -0.20$, $n = 9$). Using multiple-regression analysis with performance as the dependent variable and SWA over the right parietal cortex and arousal index as independent variables, we found a significant relationship among these 3 variables (adjusted $R^2 = 0.63$, $P = 0.02$, $n = 9$). Furthermore, the change in performance was significantly predicted by SWA over right parietal cortex ($P = 0.036$, $\beta = 0.64$) but not the arousal index ($P = 0.20$, $\beta = -0.34$).

Having established SWA, rather than arousals, as the relevant sleep variable, we then asked if some other measure of the SWS process may be more strongly correlated to changes in visuomotor performance. Running a multiple regression in which performance was the dependent variable and SWA, the number of slow waves, and slow wave energy (the cumulative SWA across a night of sleep) were independent variables, we found a significant relationship (adjusted $R^2 = 0.79$, $P = 0.01$, $n = 9$). We found that SWA ($\beta = 0.74$, $P = 0.018$) and the number of slow waves ($\beta = 0.52$, $P = 0.045$) significantly predicted the change in performance but slow wave energy did not ($\beta = -0.39$, $P = 0.11$).

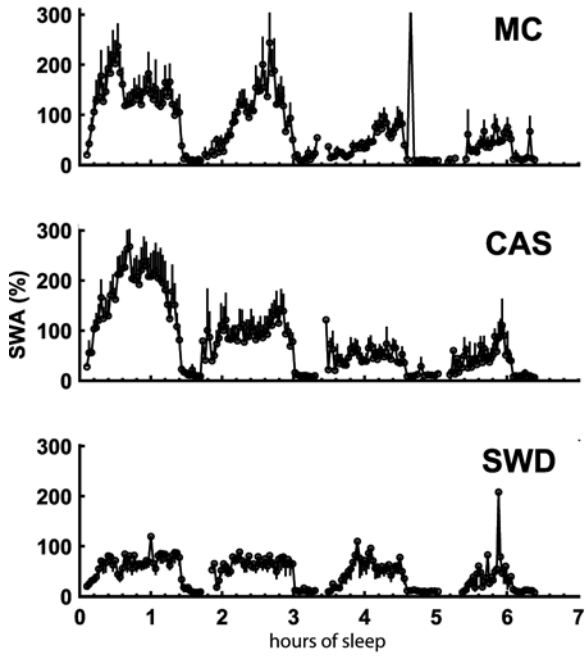

Figure 5—Time course of slow wave activity (SWA) across the night. Electroencephalographic power density for channel Fz in the 0.5- to 4.5-Hz range (n = 9, mean ± SEM) for motor control (MC), control acoustic stimulation (CAS), and slow wave deprivation (SWD). SWA values expressed as percentage of the mean SWA across the entire night in the MC condition. To account for interindividual variations in non-rapid eye movement (NREM) / rapid eye movement (REM) cycle duration, NREM episodes were subdivided into 40 bins of equal duration (percentiles), and REM episodes into 10 percentiles.

A New Automated Arousal Index Based on ERSP Analysis Characterizes the Effect of Acoustic Stimuli on the Sleep EEG

The criteria employed clinically to score spontaneous arousals[23] may be insufficiently sensitive to characterize more subtle changes in arousal state following experimental acoustic stimulation. To establish whether subtle factors in the EEG response to tones (that may not be visually evident) may be systematically different between SWD and CAS, we performed an ERSP analysis of the EEG response to auditory stimuli and used it to develop an automated arousal index. ERSP measures how strongly the mean event-related EEG power increases or decreases relative to a baseline power spectrum.[30] The advantage of this technique is that it provides a 2-dimensional image, across time and frequency, of how the EEG changes when a tone is played. Figure 9A shows average ERSP plots obtained from 3 representative subjects during SWD, CAS, and a pilot night when tones were played to induce clear behavior arousals. The latter were characterized by large prolonged increases in alpha (8- to 12-Hz) and beta/gamma (15- to 25-Hz) activity and decreases in SWA. In stark contrast, ERSP plots for SWD and CAS did not show any prolonged increase in alpha or beta/gamma activity but still showed a clear change in the EEG, best described as the occurrence of a K-complex in the 1- to 5-Hz range, lasting for 1 to 2 seconds. In SWD, there was also a clear decrease in power in the 0.5- to 5-Hz and 12- to 15-Hz ranges approximately 3 seconds after the tone. Indeed, when ERSP plots were averaged across all 9 subjects (Figure 8B), the only significant difference in SWD relative to CAS was a decrease in the EEG power encompassing the SWA range (0.5-4.5 Hz) ($P < 0.02$, 2-tailed bootstrap analysis).

Using the ERSP values for CAS and SWD in the time window of 0 to 7 seconds after the tone, and the frequency range between 0.25 and 25 Hz, a candidate set of 126 maximally informative "features" across various time and spectral windows was generated by combining all possible (integral) time windows with 6 frequency bands (0.5- to 4.5-Hz, 4- to 8-Hz, 8- to 12-Hz, 12- to 15-Hz, 20- to 24-Hz, and 16- to 25-Hz). Using this candidate set, we constructed a decision tree using the Gini diversity index[43] as the splitting criterion to distinguish between arousals and nonarousals to obtain optimal "arousal" features. The decision tree yielded 4 optimal features (ERSP 16-25 Hz, 4-7 s; 4-8 Hz, 3-6 s; 16-25Hz, 3-7 s; and 8-12 Hz, 4-7 s), and principal component analysis confirmed that there was no redundancy among features. These 4 features predicted with high accuracy (95% with 10-fold cross validation) whether an arousal as clinically defined[23] occurred in response to a tone.

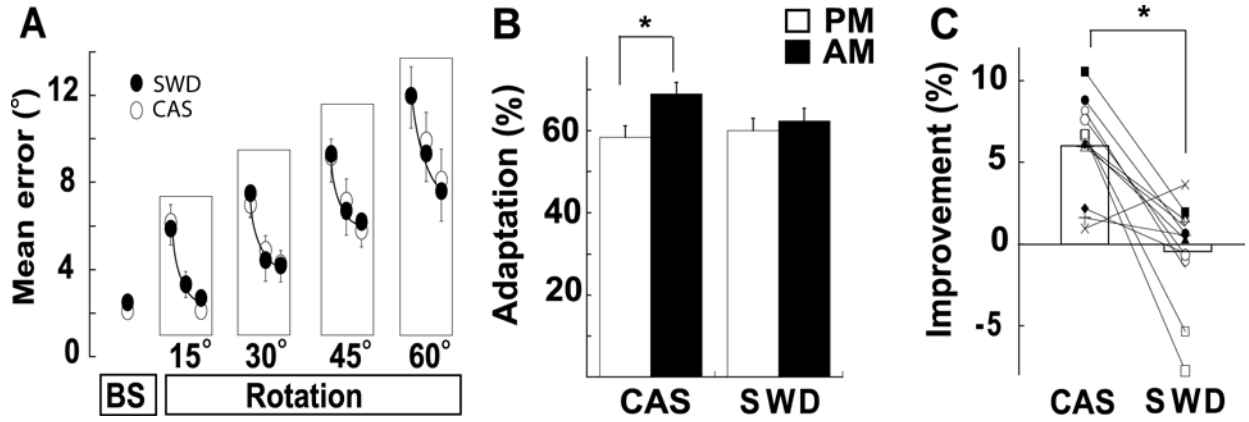

Figure 6—Behavior changes. (A) Learning curves for the rotation adaptation task for the control acoustic stimulation (CAS) and slow wave deprivation (SWD) conditions in the evening. The mean directional error for each block of 90 movements is plotted. Points are means across subjects and bars represent standard errors (n = 12). (B) Amount of adaptation for CAS and SWD. Mean directional error was tested in the evening at the end of training using an imposed rotation of 60º. Subjects were then retested with the same imposed rotation of 60º in the morning. Adaptation computed as a percentage with the formula: 100 *(1-[mean directional error / 60°]) represents the amount to which the subject was able to adapt to an imposed rotation (n = 12, mean ± SEM). (C) Change in individual postsleep visuomotor performance on rotation-adaptation task. Values represent percentage of improvement in mean directional error upon retesting the next morning relative to testing the previous evening (n = 12, mean ± SEM, paired *t* test, P = 0.0007)

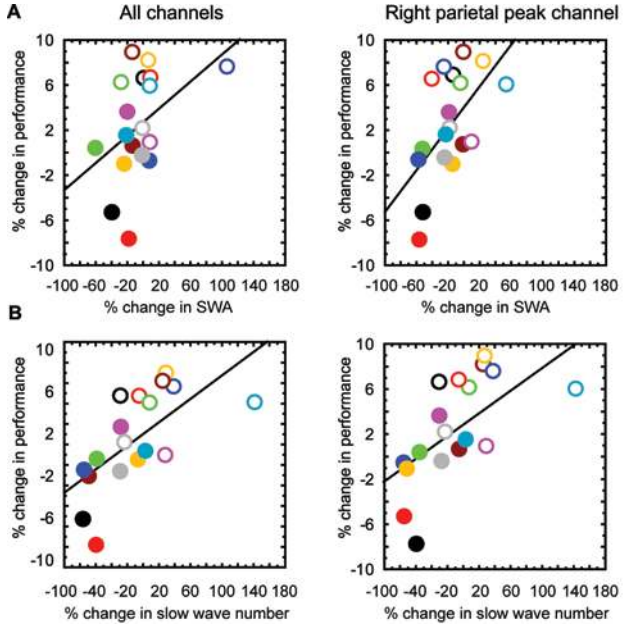

Figure 7—Relationship between sleep parameters and postsleep performance. (A) Relationship between change in slow wave activity (SWA) (% of motor control [MC]) and change in postsleep visuomotor performance for all channels and a single channel over right parietal cortex. Each subject is represented by a different color (filled symbols: slow wave deprivation [SWD]; empty symbols: control acoustic stimulation [CAS]). Solid lines indicate a regression line. (B) Relationship between the change in number of slow waves (% of MC) and change in postsleep visuomotor performance for all channels and a single channel over right parietal cortex.

Next, we used these maximally informative, "arousal" sensitive features to automatically categorize the tone-induced effects on the sleep EEG. Using the affinity propagation clustering algorithm,[44] we found that 87% of the responses to tones clustered into 16 clusters (Supplementary Figure 2A; see *SLEEP* website at www.journalsleep.org). Intriguingly, 72.2% of all tones that led to an arousal fell in 4 clusters (1, 11, 15, 16; Supplementary Figure 2A). Moreover, within these 4 clusters, the tones resulting in arousals made up, on average, 9.4% of all tones per cluster, compared with 1.0% in the other 12 clusters. Thus, this analysis shows that (1) arousals can be defined with high accuracy, in a fully automated manner, on the basis of the ERSP; (2) arousals do indeed cluster differently from nonarousals; and (3) a very large number of EEG responses to tones that were not visually recognized as arousals clustered together with arousals as clinically defined,[23] raising the issue whether they may represent a more subtle kind of sleep fragmentation. Next, we quantified how the EEG effects of CAS and SWD were distributed across the 16 clusters (Supplementary Figure 2B). Using the similarity distance measure[45] between all clusters (value: 0.24) indicated a different distribution; specifically, clusters 11 and 15 were significantly different between the SWD and CAS conditions ($P < 0.05$, n = 9, paired *t* test). That is, SWD and CAS tones produced different EEG responses as evaluated by automated clustering.

Finally, to establish whether the differences in the effects of the tones during the SWD and the CAS night—as evaluated by the automated arousal index—could account for their different effects on performance, we performed multiple-regression analysis using visuomotor performance as the dependent variable and SWA over the right parietal cortex and the number of events in CAS and SWD for clusters 11 and 15 as independent variables. We found a significant relationship between these variables (adjusted $R^2 = 0.52$, P = 0.04), which showed that the change in performance was significantly predicted by SWA over right parietal cortex (P = 0.02, $\beta = 0.80$, n = 9) and not by the number of events in "arousal" clusters 11 and 15 (cluster 11: P = 0.46, $\beta = -0.26$; cluster 15: P = 0.93, $\beta = 0.03$). Thus, these results confirm that the decrements in visuomotor performance after SWD cannot be accounted for by a "lightening" of the EEG or by sleep fragmentation but, rather, by a decrease in SWA.

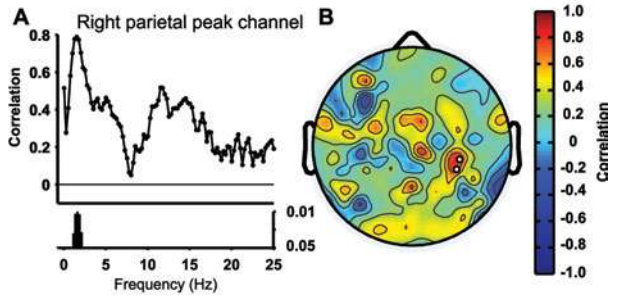

Figure 8—Correlation between performance and electroencephalographic (EEG) power. (A) Correlation between EEG power (0.25 Hz bins; normalized to motor control [MC]) and visuomotor performance change during slow wave deprivation (SWD) for a single channel over right parietal cortex. Black bars indicate frequency bins with significant correlation (n = 9, P < 0.05, paired *t* test). **(B)** Topographic distribution of the correlation between SWA (normalized to MC) and visuomotor performance changes after SWD. White dots represent electrodes that were significantly correlated (n = 9, P < 0.05, paired *t* test). Values were plotted at the corresponding position on the planar projection of the scalp surface, and interpolated (biharmonic spline) between electrodes using EEGLAB.[27]

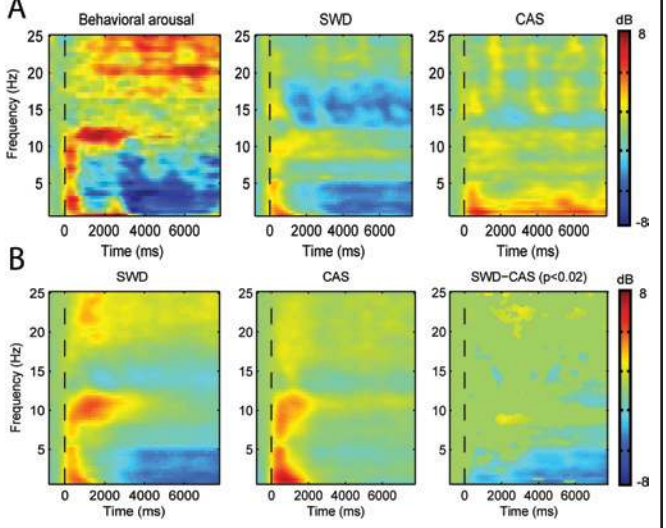

Figure 9—Event-related spectral perturbation (ERSP) analysis for slow wave deprivation (SWD) and control acoustic stimulation (CAS). (A) Representative ERSP plots obtained from 3 representative subjects. Left panel shows a pilot night when tones were played to induce a behavioral arousal. (B) Mean change in electroencephalographic (EEG) power (n = 9) 0-7 seconds after the delivery of the tone (vertical dashed lines) for SWD and CAS. Right panel shows significant differences in ERSP between the 2 conditions (P < 0.02, 2-tailed bootstrap EEGLAB[27]).

DISCUSSION

Unlike previous reports,[16-19] we evaluated the effects of SWD relative to a control condition during which a similar number of tones was delivered during NREM sleep periods showing little SWA. This control rules out aspecific effects of auditory stimulation during sleep that might have interfered with memory consolidation irrespective of the effects of SWD. As shown here, visuomotor performance did improve after CAS but failed to do so after SWD, demonstrating that the auditory stimulation per se cannot account for the lack of improvement. On the other hand, although subjects in the CAS condition improved after sleep, they did so less than previously described in undisturbed subjects[11] (6% vs 11%), and the SWA increase over right parietal cortex in CAS relative to MC did not reach significance. Therefore, auditory stimulation may have a slight impact on its own, perhaps due to lightening of sleep[46] or modulation of autonomic[47] and neuroendocrine functions.[48] Alternatively, because CAS produced a net decrease in the EEG power between 0.25 and 1.25 Hz—compared with MC (though it failed to reach significance, see Figure 3)—even a slight reduction of sleep SWA may have detrimental effects on visuomotor performance.

In agreement with our previous findings,[11] we found here that the change in visuomotor performance after SWD was positively correlated with changes in SWA but not with spindle power. On the other hand, SWD showed a significant increase in spindle density, compared with MC, suggesting that acoustic stimulation may increase spindle activity.[49] Apparently, the increase in spindle activity associated with auditory stimulation did not benefit visuomotor performance. Several studies have shown a general correlation between learning and spindle activity,[37] and, in at least some tasks, within-subject performance improvements after learning correlated with increases in spindle activity.[32-37] These findings, however, were correlative, and only 3 studies so far have examined SWA, spindle activity, and postsleep performance after learning the same task. Our previous findings (visuomotor)[11] and a similar study (perceptual learning)[18] found that postsleep performance was positively correlated with SWA but not with spindle power. Another study[35] showed an increase in both SWA and spindle power (low range) after a word-pair learning task, but performance correlated positively only with the latter. However, only 8 EEG electrodes were used, and a topographically specific correlation between SWA and performance may have been missed. The relationship between slow waves and spindles is complex—for instance, in the course of a sleep cycle, there is an inverse relationship between epochs rich in SWA and epochs rich in spindles,[8] yet it is well established that individual slow waves trigger spindles.[50] To determine whether inconsistencies across studies reflect technical problems or true physiologic differences, further work will need to assess the topography of learning effects using high-density EEG and to establish causality by selective slow wave versus spindle suppression (or enhancement).

Previous studies have shown that sleep fragmentation can lead to impaired cognitive performance and excessive daytime sleepiness.[23] In the present study, the number of clinically defined arousals,[23] though small, was higher in SWD than in CAS, but the increase in arousal index could not account for the decrements in visuomotor performance after SWD. To ascertain that it was the suppression of SWA, and not some tone-induced, short-lasting "lightening" of the EEG not captured by the commonly used arousal criteria, that was responsible for the effects of SWD on performance, we used ERSP analysis and developed a novel multivariate method for automatically categorizing stimulus-induced EEG changes in an unbiased manner. This analysis (1) confirmed that, as intended, the only significant difference between the effects on sleep EEG during SWD and CAS was a decrease in SWA after SWD; (2) demon-

strated that the EEG changes produced by tones during sleep (SWD or CAS) are very different from those associated with a behavior arousal; (3) showed that tone-induced EEG perturbations can be categorized into 16 distinct clusters, the distribution of which is different for SWD and CAS; (4) confirmed that the negative effects on performance after SWD are not due to subtle "arousal" effects, as reflected in the different distribution across clusters, but to a decrease in SWA.

The main result of the present study is the demonstration that a local increase in slow waves is not merely a correlate of learning but that slow waves themselves play a causal role in sleep-dependent enhancements in visuomotor performance. This conclusion is supported by the results of a recent study[18] showing that slow-wave suppression using acoustic stimuli also prevents the postsleep improvement in performance after visual texture discrimination learning. Although the latter study did not control for the effects of acoustic stimulation per se, the improvement after sleep was positively correlated with SWA and negatively correlated with spindle power. Together, the present study and that of Aeschbach et al[18] make a strong case that interfering with sleep slow waves prevents sleep-dependent improvements even though sleep is not reduced. Our study suggests, in addition, that both SWA and the number of slow waves were positively correlated with visuomotor performance. The correlation was stronger with SWA, suggesting that even small waves, not counted by the slow-wave detection algorithm,[26] may contribute to improvements in visuomotor performance.

Other studies also suggest that increasing SWA may have a beneficial effect on performance. In 1 study, intermittent transcranial direct current stimulation during early NREM sleep (which temporarily increased SWA for 3 minutes) was followed by enhanced retention in a paired-associate memory task.[51] Two other reports showed that pharmacologic enhancement of SWS reduces cognitive impairments associated with sleep restriction,[52,53] though memory consolidation was not assessed. Taken as a whole, the results of these studies have several implications. First, slow waves appear to be more than just an epiphenomenon of NREM sleep by causally mediating some of the beneficial effects of sleep. Second, slow waves appear to benefit not just 1 aspect, but several aspects, of performance, from the prevention of cognitive impairments to memory consolidation. Third, slow waves appear to benefit more than 1 kind of memory, from declarative learning[51] to perceptual learning,[18] to skill learning (this study).

How do slow waves cause an improvement in performance the next day? A first possibility is that information acquired during wakefulness is reactivated during sleep, leading to further potentiation of synaptic circuits involved in learning and thereby to memory consolidation.[1] This hypothesis is supported by multiunit recordings in animals showing off-line reactivation of neurons and brain areas involved in the initial learning.[54,55] Furthermore, a recent neuroimaging study showed that reexposure to an odor during sleep reactivates a memory and leads to improved retention in an object-location memory task.[56] However, direct evidence for synaptic potentiation during sleep is still lacking.

A second possibility is that sleep slow waves downscale synaptic strength to a baseline level that restores optimal conditions for energy consumption, space occupancy, cellular supplies, and desaturation of the ability to learn. Moreover, downscaling would lead to an increase in signal-to-noise ratios that could result in performance improvements.[57] This last prediction has recently been validated in a combined modeling/behavior study that also employed a rotation-adaptation task.[58] In the model, implementing rotation learning through a local increase in synaptic strength, and the effects of sleep as a proportional downscaling of all synapses, led to an improvement in the model's performance that closely mirrored the results obtained in parallel behavior experiments. By contrast, modeling the effects of sleep as additional strengthening of synapses did not fit the behavior results. Additional modeling of sleep dynamics in large-scale thalamocortical circuits has shown that a local increase in SWA, as observed in Huber et al[11] after rotation learning—and the global decrease in SWA that occurs during sleep—can be accounted for, respectively, by a wake-related strengthening of synapses and by a sleep-related global downscaling.[7] The simulations show that changes in synaptic strength lead to corresponding changes in neural synchronization and, thereby, in the amplitude of sleep slow waves, consistent with evidence in both rats[59] and humans.[26] Finally, recent rat experiments have confirmed that both molecular and electrophysiologic indicators of synaptic strength increase during wakefulness and decline in the course of sleep and that they do so in a way that is correlated with SWA.[60] Altogether, these mechanisms can account in a parsimonious manner for the observed correlation between learning and SWA as well as for the beneficial effects of slow waves on performance.

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REFERENCES

1. Born J, Rasch B, Gais S. Sleep to remember. Neuroscientist 2006;12:410-424.
2. Giuditta A, Ambrosini MV, Montagnese P, et al. The sequential hypothesis of the function of sleep. Behav Brain Res 1995;69:157-66.
3. Stickgold R, Whidbee D, Schirmer B, Patel V, Hobson JA. Visual discrimination task improvement: A multi-step process occurring during sleep. J Cogn Neurosci 2000;12:246-54.
4. Plihal W, Born J. Effects of early and late nocturnal sleep on declarative and procedural memory. J Cogn Neurosci 1997;9:534-47.

5. Steriade M. The corticothalamic system in sleep. Front Biosci 2003;8:D878-99.
6. Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G. The sleep slow oscillation as a traveling wave. J Neurosci 2004;24:6862-70.
7. Esser SK, Hill SL, Tononi G. Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. Sleep 2007;30:1617-30.
8. Dijk DJ, Hayes B, Czeisler CA. Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. Brain Res 1993;626:190-9.
9. Werth E, Dijk DJ, Achermann P, Borbély AA. Dynamics of the sleep EEG after an early evening nap: experimental data and simulations. Am J Physiol 1996;271:R501-10.
10. Knoblauch V, Kräuchi K, Renz C, Wirz-Justice A, Cajochen C. Homeostatic control of slow-wave and spindle frequency activity during human sleep: effect of differential sleep pressure and brain topography. Cereb Cortex 2002;12:1092-100.
11. Huber R, Ghilardi MF, Massimini M, Tononi G. Local sleep and learning. Nature 2004;430:78-81.
12. Ghilardi M, Ghez C, Dhawan V, Moeller J, et al. Patterns of regional brain activation associated with different forms of motor learning. Brain Res 2000;871:127-45.
13. Krakauer JW, Ghez C, Ghilardi MF. Adaptation to visuomotor transformations: consolidation, interference, and forgetting. J Neurosci 2005;25:473-8.
14. Marinelli L, Crupi D, Di Rocco A, et al. Learning and consolidation of visuo-motor adaptation in Parkinson's disease. Parkinsonism Relat Disord. 2009;15:6-11.
15. Ghilardi MF, Silvestri G, Feignin A, et al. Implicit and explicit aspects of sequence learning in pre-symptomatic Huntington's disease. Parkinsonism Relat Disord 2008;14:457-64.
16. Dijk DJ, Beersma DG. Effects of SWS deprivation on subsequent EEG power density and spontaneous sleep duration. Electroencephalography and Clinical Neurophysiology 1989;72:312-20.
17. Ferrara M, De Gennaro L, Bertini M. Selective slow-wave sleep (SWS) deprivation and SWS rebound: do we need a fixed SWS amount per night? Sleep Res Online 1999;2:15-9.
18. Aeschbach D, Cutler AJ, Ronda JM. A role for non-rapid-eye-movement sleep homeostasis in perceptual learning. J Neurosci 2008;28:2766-72.
19. Tasali E, Leproult R, Ehrmann DA, Van Cauter E. Slow-wave sleep and the risk of type 2 diabetes in humans. Proc Natl Acad Sci U S A. 2008;105:1044-9.
20. Neckelmann D, Ursin R. Sleep stages and EEG power spectrum in relation to acoustical stimulus arousal threshold in the rat. Sleep 1993;16:467-77.
21. Rosenthal L, Bishop C, Helmus T, Krstevska S, Roehrs T, Roth T. Auditory awakening thresholds in sleepy and alert individuals. Sleep 1996;19:2905.
22. Rechtschaffen A, Kales A. A manual of standardized terminology, techniques, and scoring system for sleep stages of human subjects. UCLA, Los Angeles: Brain Information Service/Brain Research Institute, 1968.
23. Bonnet MH, Doghramji K, Roehrs T, et al. The scoring of arousal in sleep: reliability, validity, and alternatives. J Clin Sleep Med 2007;3:133-45.
24. Bonnet MH, Arand H. EEG arousal norms by age. J Clin Sleep Med 2007;3:271-4.
25. Huber R, Ghilardi MF, Massimini M, et al. Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat Neurosci 2006;9:1169-76.
26. Riedner BA, Vyazovsky VV, Huber R, et al. Sleep homeostasis and cortical synchronization: III. a high-density EEG study of sleep slow waves in humans. Sleep 2007;30:1643-57.
27. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 2004;134:9-21.
28. Herscovitch J, Broughton R. Sensitivity of the Stanford sleepiness scale to the effects of cumulative partial sleep deprivation and recovery oversleeping. Sleep 1981;4:83-91.
29. Dinges DF, Pack F, Williams K, et al. Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. Sleep 1997;20:267-77.
30. Makeig S. Auditory event-related dynamics of the EEG spectrum and effects of exposure to tones. Electroencephalogr Clin Neurophysiol 1993;86:283-93.
31. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 2002;15:1-25.
32. Gais S, Molle M, Helms K, Born J. Learning-dependent increases in sleep spindle density. J Neurosci 2002;22:6830-4.
33. Clemens Z, Fabo D, Halasz P. Overnight verbal memory retention correlates with the number of sleep spindles. Neuroscience 2005;132:529-35.
34. Clemens Z, Fabo D, Halasz P. Twenty-four hours retention of visuospatial memory correlates with the number of parietal sleep spindles. Neurosci Lett 2006;403:52-6.
35. Schmidt C, Peigneux P, Muto V, et al. Encoding difficulty promotes postlearning changes in sleep spindle activity during napping. J Neurosci 2006;26:8976-82.
36. Nishida M, Walker MP. Daytime naps, motor memory consolidation and regionally specific sleep spindles. PLoS ONE 2007;2:e341.
37. Schabus M, Hoedlmoser K, Pecherstorfer T, et al. Interindividual sleep spindle differences and their relation to learning-related enhancements. Brain Res 2008;1191:127-35.
38. Werth E, Achermann P, Borbély AA. Fronto-occipital EEG power gradients in human sleep. J Sleep Res 1997;6:102-12.
39. Cajochen C, Foy R, Dijk DJ. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. Sleep Res Online 1999;2:65-9.
40. Finelli LA, Borbely AA, Achermann P. Functional topography of the human nonREM sleep electroencephalogram. Eur J Neurosci 2001;13:2282-90.
41. Finelli LA, Achermann P, Borbely AA. Individual 'fingerprints' in human sleep EEG topography. Neuropsychopharmacology 2001;25:S57-62.
42. De Gennaro L, Ferrara M, Vecchio F, Curcio G, Bertini M. An electroencephalographic fingerprint of human sleep. Neuroimage 2005;26:114-22.
43. Gini, Corrado (1912). "Variabilit e mutabilit" Reprinted in Memorie di metodologica statistica (Ed. Pizetti E, Salvemini, T). Rome:Libreria Eredi Virgilio Veschi (1955).
44. Frey JF, Dueck D. Clustering by passing messages between data points. Science 2007;315:972-6.
45. Coen MH. Multimodal dynamics: self-supervised learning in perceptual and motor systems. The Massachusetts Institute of Technology. 2007 Ph.D. Dissertation.
46. Muzet A. Environmental noise, sleep and health. Sleep Med Rev 2007;11:135-42.
47. Carter NL, Hunyor SN, Crawford G, Kelly D, Smith AJ. Environmental noise and sleep--a study of arousals, cardiac arrhythmia and urinary catecholamines. Sleep 1994;17:298-307.
48. Maschke C, Hecht K. Stress hormones and sleep disturbances - electrophysiological and hormonal aspects. Noise Health 2004;6:49-54.
49. Sato Y, Fukuoka Y, Minamitani H, Honda K. Sensory stimulation triggers spindles during sleep stage 2. Sleep 2007;30:511-8.

50. Steriade M. Grouping of brain rhythms in corticothalamic systems. Neuroscience 2006;137:1087-106.
51. Marshall L, Helgadottir H, Molle M, Born J. Boosting slow oscillations during sleep potentiates memory. Nature 2006;444:610-3.
52. Walsh JK, Deacon S, Dijk DJ, Lundahl J. The selective extrasynaptic GABAA agonist, gaboxadol, improves traditional hypnotic efficacy measures and enhances slow wave activity in a model of transient insomnia. Sleep 2007;30:593-602.
53. Walsh JK, Randazzo AC, Stone K, et al. Tiagabine is associated with sustained attention during sleep restriction: evidence for the value of slow-wave sleep enhancement? Sleep 2006;29:433-43.
54. Wilson MA, McNaughton BL. Reactivation of hippocampal ensemble memories during sleep. Science 1994;265:676-9.
55. Skaggs WE, McNaughton BL. Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. Science 1996;271:1870-3.
56. Rasch B, Buchel C, Gais S, Born J. Odor cues during slow-wave sleep prompt declarative memory consolidation. Science 2007;315:1426-9.
57. Tononi G, Cirelli C. Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull 2003;62:143-50.
58. Hill S, Tononi G, Ghilardi MF. Sleep improves the variability of motor performance. Brain Research Bulletin 2008;76:605-11.
59. Vyazovskiy VV, Riedner BA, Cirelli C, Tononi G. Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. Sleep 2007;30:1631-42.
60. Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 2008;11:200-8.