Slow Wave Sleep and REM Sleep Awakenings Do Not Affect Sleep Dependent Memory Consolidation

Lisa Genzel[1]; Martin Dresler, MA, MSc[1]; Renate Wehrle, PhD[1]; Michael Grözinger, MD[2]; Axel Steiger, MD[1]

[1]Max Planck Institute of Psychiatry, Munich, Germany; [2]Department of Psychiatry, University Clinic Aachen, Aachen, Germany

Study Objectives: The effects of REM sleep and slow wave sleep (SWS) deprivation on sleep-dependent motor and declarative memory consolidation.

Design: Randomized, within-subject, cross-over study

Setting: Weekly (women: monthly) sleep laboratory visits, with retest 60 hours later

Participants: Twelve healthy subjects (6 men) aged between 20 and 30 years

Interventions: REM sleep deprivation, SWS deprivation, or undisturbed sleep

Measurements and Results: We deprived subjects once each of REM sleep and SWS, and once let them sleep undisturbed through the night. After each night, we tested declarative and procedural memory consolidation. We tested memory performance by a verbal paired associate task and a sequential finger-tapping task at 21:00 on the study night and again 60 hours later. Although REM sleep and SWS awakenings led to a significant reduction of the respective sleep stages, memory consolidation remained unaffected. We also found a significant correlation between the declarative task and sleep spindles in the undisturbed condition, especially the sleep spindles in the first third of the night.

Conclusion: We suggest that word-pair learning relies on stage 2 sleep spindles and requires little SWS. Their sleep dependent consolidation is not affected by SWS deprivation. Simple motor tasks may either be consolidated in stage 2 sleep or depend on only small amounts of REM sleep. Their sleep dependent consolidation is not influenced by REM sleep deprivation.

Keywords: Memory, REM sleep deprivation, slow wave sleep deprivation, motor learning, declarative learning, sleep spindles

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THERE IS GROWING AMOUNT OF EVIDENCE THAT MEMORY CONSOLIDATION IS CONNECTED TO SLEEP. SLEEP DEPENDENT MEMORY CONSOLIDATION HAS been shown in humans for declarative as well as non-declarative material.[1,2] It is less clear which sleep stages or electroencephalographic microstructures are relevant for different learning tasks.[3-7]

The relevant sleep structures have been studied mostly by indirect means. Some concepts are based on the observation of changes of the sleep structure after learning experiences. For example, for a procedural motor learning task a correlation between the performance improvement and the amount of stage 2 sleep was shown.[2] The amount of REM sleep appears to be increased after a motor-learning task.[8]

Other studies have taken advantage of the uneven distribution of sleep stages over the 2 halves of a night. Declarative memory consolidation was associated with the first half of the night, when SWS is known to be most prominent. In contrast, procedural memory tasks were connected with the second half of the night when REM sleep dominates.[9] The associations with the specific sleep stages were deduced from the experiment even though all sleep stages were present in both halves of the night, and therefore remain quite speculative.

There have been contradictory findings on the verbal paired associates task,[9] which tests declarative memory. Some results favor a connection with SWS,[9-12] others a REM sleep dependency.[13-15] One explanation is that small differences in the degree of difficulty or emotionality can have a large influence on the outcome.[4,16] It is not only emotionality and difficulty that affect sleep-dependent memory consolidation; no task is process pure, meaning that all tasks have different explicit and implicit demands. This is presumably reflected by different sleep mechanisms being associated with memory consolidation.

Small differences in test design can cause large discrepancies in the study results of the sleep dependence of memory processes. This may explain why some results are regularly found only by some groups. As a consequence, clear and consistent test design is even more important.

It has also been found that REM sleep deprivation impairs consolidation of some procedural tasks but had no effect on declarative memory consolidation.[4] A few more recent studies have utilized selective REM sleep deprivation (REMD).[17,18] However, there have been no studies comparing SWS deprivation (SWSD) with REMD with respect to motor and declarative memory consolidation. Selective sleep deprivation has been criticized for evoking additional unspecific effects like arousal, emotional irritation, and concentration deficits. Therefore we chose 2 different deprivation conditions as additional controls and measured stress, concentration, and sleepiness throughout the experiment. Selective sleep deprivation appears to be the most direct method to investigate the impact of specific sleep stages on memory consolidation.

Taken together, most experimental results suggest a connection between procedural memory and REM sleep; declarative memory appears to be dependent on SWS, even though numerous studies found no direct relationships between word-pair recall and SWS.[19] The aim of the present study was to clarify the

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Address correspondence to: Lisa Genzel, Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, 80804 Munich, Germany; Tel: 0049 89 306 22 386 Fax: 0049 89 306 22 552; E-mail: genzel@mpipsykl.mpg.de

link between specific sleep stages and different types of memory consolidation; this was done by suppressing sleep stages associated with motor and declarative memory consolidation within subjects.

EXPERIMENTAL DESIGN AND METHODOLOGY

Subjects

The experimental subjects were healthy volunteers (n = 12; 6 males and 6 females), aged 20-30 years. They were all university students and were paid for the participation in the study. We first screened the subjects for psychiatric, physical, and sleep disorders by history and physical examination. We also performed an electroencephalogram, electrocardiogram, urinary drug screening, and routine blood examination on each subject. Exclusion criteria were shift-work at night, a transmeridian flight during the last year, substance abuse, medicinal treatment in the last 3 months, professional piano playing (> 5 years intensive training), and professional typewriting. We also screened subjects for sleep disorders during their first adaptation night in the sleep laboratory.

The subjects were randomly assigned to one of 6 different experimental groups, with a different succession of the deprivation nights. The subjects were blind to the experimental condition of the study nights. They were not aware of the existence of the undisturbed night and expected 3 deprivation nights. We chose a within-subject design, since neurobehavioral deficits and sleep physiology during and after sleep deprivation are trait-like and exhibit robust interindividual differences.[20,21]

Participants agreed to have regular sleep patterns throughout the experiment. The ethics committee of Ludwig Maximilian University Faculty of Medicine, Munich, approved the research project.

Polysomnographic Recording Parameters

We recorded, stored, and analyzed polysomnograms with a digital recorder (Comlab 32 Digital Sleep Lab, Brainlab V 3.3 Software, Schwarzer GmbH, Munich, Germany). Polysomnograms were conducted for the first of the 3 adaptation nights and the 3 study nights (control, SWSD, REMD). We recorded EEG with C3 and C4 leads (filtered from 0.5 to 70 Hz), electrooculogram (EOG), and mental/submental electromyogram (EMG), with a sampling rate of 250 Hz.

Procedures

The subjects underwent 3 experimental sessions. Each session consisted of an adaptation night, a study night, and a retest at 11:00 after 2 nights of recovery sleep. On the adaptation night the participants arrived at 22:00. The electrodes were placed immediately, and the participants could sleep undisturbed from 23:00 to 07:00. On the study night, the subjects were required to arrive at the sleep laboratory at 21:00, when they completed the D2-Concentration test (D2)[22] and the Stanford Sleepiness Scale (SSS),[23] followed by the learning phase of the verbal paired associates task[9] and the finger tapping task.[2] We conducted these tasks in a randomized order to avoid a confounding effect of a reciprocal interaction between the tasks.[24] Subsequently the electrodes were placed, the lights were turned off at 23:00, and the subjects underwent SWSD, REMD, or an undisturbed night with no awakenings. The expectancy to be woken many times at night is a strong stressor, which may inhibit evening learning. To counteract this effect we also included an undisturbed condition, during which the subjects expected awakenings. The final awakening was at 07:00, followed by the completion of the D2 test, the SSS, and a stress scale referencing the study night. For the stress scale, the subjects were asked to pick a number between 1 "not at all stressful" and 10 "very stressful" according how stressful the night seemed to them. The subjects were forbidden to sleep before 22:00 on the day after the deprivation night, to avoid an early relapse into the deprived sleep stage. We did not use actigraphy. We told the subjects to refrain from rehearsal of the tests and to keep a regular sleep pattern during the whole experiment. The subjects spent 2 nights of recovery sleep at home to avoid immediate effects of the sleep disruptions on test outcomes.[25] The participants returned at 11:00 to fill out the D2 and the SSS, and to undergo retesting in a randomized order of the verbal paired associates task and the finger-tapping task. This was possible since it has been shown that only the first night after learning is significant for sleep-dependent consolidation and cannot be compensated by the following nights.[25] With an interval $\geq$ 1 week,[17] subjects participated in the other 2 experimental sessions (mean male 15.42 ± 6.13 d; mean female 28.9 ± 4.36 d). The sessions of the female participants always took place in the first week of their menstrual cycle to avoid hormonal influences on sleep and learning.[26-28] Each subject had one night with SWSD, one night with REMD, and one undisturbed night, balanced to avoid sequence effects.

Learning Tasks

All subjects learned 2 tasks, one using declarative memory and one using procedural memory. We employed a sequential finger tapping task[2,29] as a tool of procedural memory analysis. This task required subjects to press 4 numeric keys on an altered computer keyboard with the non-dominant hand, repeating the 5-element sequence as quickly and accurately as possible for a period of 30 s. The numeric sequence changed on every experimental condition and was displayed on the screen to exclude any working memory component to the task. A white dot appeared on the screen under the sequence for every pressed key. The computer recorded and stored all keystroke responses. For each 30-s trial, the computer noted the number of complete sequences achieved, the number of errors made, and the number of correct sequences typed. Training consisted of twelve 30-s trials interrupted by 20-s rest periods. At retest, the subjects completed 3 trials. The score of the correctly tapped sequences included accuracy and speed performance. We used the average score from the last 3 trials of the training condition as end-training performance and the average score from the 3 trials of the retesting as consolidation performance. To measure sleep-dependent consolidation, we divided the retest performance by the end-training performance, which represents the relative percentile improvement of speed and accuracy (motor consolidation; mcons; presented in * 100%).

For the study of declarative memory, we employed a paired associates learning task.[9] We used 3 parallel word lists for the measurements on each of the 3 nights. Each list consisted of 28 German noun-pairs, balanced across word lists in emotionality, meaningfulness, and concreteness, with 2 additional dummy pairs at the beginning and end to buffer primacy and recency effects.[9] In the learning condition, the word-pairs were first presented to the subject for 5 s each; immediately thereafter a cued recall followed, in which the subject had to type the matching noun after being shown the first word of the pair. If the participant was not able to recall the right word, the correct answer was displayed. Cued recall was repeated until the subject had achieved 60% (= 17 word pairs) correct responses.[9]

In the retest condition, the list was recalled once, and the number of correctly known word pairs was measured. At the training and retest condition, the subject had unlimited time to respond to the cued recall. The retest performance divided by the training performance represents the sleep-dependent consolidation measure (declarative consolidation; dcons presented as * 100%). Dcons constitutes the relative percentile change in declarative memory.

Sleep Deprivation Protocol

The sleep deprivation was accomplished by direct visual scoring. In the REMD condition, we woke the subjects as soon as these 3 criteria were present for ≥ 30 s: (1) a desynchronized EEG; (2) low amplitude in the EMG; (3) no sleep spindles or K-complexes. The occurrence of rapid eye movements were not obligatory, since according to Rechtschaffen and Kales, they require a retrograde classification.[30,31]

On the SWSD condition we woke the subjects as soon as there were 2 delta waves apparent in a 30-s EEG recording.

For the awakening, the experimenter switched on a dim red light (to avoid influence on the circadian system by bright light) and stepped into the room. The subjects did simple arithmetic multiplications for 2 min, after which they were allowed to sleep again. We chose a 2-min arousal, since after shorter awakenings subjects normally return immediately to the deprived sleep stage, and longer arousals have a too strong an effect on the total sleep time.[30,32]

Sleep Data Analysis

For sleep data analysis, independent professional scorers scored the sleep stages using standard criteria.[31] In addition, EEG from the experimental nights underwent spectral analysis through a fast Fourier transformation, using in-house software. The EEG was digitally filtered from 0.53 to 30 Hz (24 dB/octave) after visually identified EEG artifacts had been carefully removed. Power spectra were derived from a 2-s window, shifted for 1 s, and averaged per 30-s epoch. Frequency bands (based on summed power values per 2-s window) were calculated for delta (0.53–4 Hz), theta (4.5–8 Hz), alpha (8.5–12 Hz), sigma (12.5–16 Hz), and beta (16.5–20 Hz) frequency ranges in all sleep stages per night. An automated algorithm detected the sleep spindles. The algorithm factored in minimal amplitude, spindle duration, and frequency range and provided sleep spindle features such as the number, duration, amplitude, and frequency.[33-35] Analyzed parameters were spindle density (SpD) and absolute spindle activity (aSpA; absolute number of spindles per night × mean spindle amplitude × mean spindle duration) in sleep stage 2 and NREM. We used aSpA since it incorporates the spindle activity (mean spindle amplitude × mean spindle duration), which reflects the intensity of the spindle process,[34-36] and absolute number of sleep spindles.

Statistical Analyses

For the statistical analysis, we first partitioned the variables in 5 sets and then performed for each of them a multivariate analysis of variance (MANOVA) with repeated measures design: one for sleep stages (S2, SWS, REM, Wake), one for spectral frequency bands (delta, theta, alpha, sigma, beta), one for sleep spindle measures (SpD, aSpA), one for alertness data (Stress, SSS in the evening, morning, and at retest, D2 in the evening, morning, and at retest), and one for learning data (first tapping trial, tapping end training performance, tapping retest performance, mcons, dcons). The only influential factor in the MANOVAs was the experimental condition, a within-subjects factor with 3 levels (REMD, SWSD, undisturbed night). For variable sets that revealed a significant factor effect, we conducted further univariate F-tests to identify those variables on which the factor effect was significant. Variables tests with contrasts were subsequently performed to locate significant differences between the factor levels.

Since sleep spindles have often been correlated with memory consolidation,[35-44] we performed a bivariate Pearson correlation between the declarative consolidation (dcons) and the stage 2 and NREM spindle measures (SpD and aSpA), as well as between the motor consolidation (mcons) and the spindle measures. If a significant correlation was found, we carried out further correlations with aSpA and SpD of the thirds of the night. We chose a P value of 0.05 and made Bonferroni adjustments for each statistical test.

RESULTS

Sleep Measures

The MANOVA for the scores of all recorded subjects for stage 2, stage 3+4 (SWS), and stage REM showed a significant difference between experimental conditions ($F_{12,34} = 17.18$; $P < 0.001$). The univariate F-test revealed a significant difference between conditions for SWS ($F_{2,1} = 39.014$; $P < 0.001$). Further contrasts showed that SWS was significantly reduced in the SWSD condition compared to both of the other experimental conditions (undisturbed/SWSD: $t_{11} = 7.650$; $P < 0.001$; REMD/SWSD: $t_{11} = 8.900$; $P < 0.001$). In addition, the univariate F-test showed a significant difference between the sleep-recorded experimental conditions on stage REM measures ($F_{2,1} = 63.411$; $P < 0.001$). Contrasts further showed a significant reduction of REM sleep in the REMD condition in comparison to both SWSD and the undisturbed night (undisturbed/REMD: $t_{11} = 10.909$; $P < 0.001$; REMD/SWSD: $t_{11} = -9.261$; $P < 0.001$). Table 1 displays all sleep measures, number of forced awakenings, learning data, and supplementary data. The MANOVA for delta, theta, alpha, sigma, and beta spectral power of all subjects

Table 1—Sleep Stages, Supplementary Data and Learning Task Divided by Experimental Condition of the Study Night

	Undisturbed		REMD		SWSD		
	M	(SD)	M	(SD)	M	(SD)	
Stage 1 in min	26.0	(2.7)	48.4	(4.6)	42.5	(4.3)	
Stage 2 in min	190.5	(12.8)	192.5	(7.3)	204.5	(6.8)	
SWS in min	105.2	(9.7)	90.7	(7.8)	26.5*	(3.1)	undisturbed/SWSD: $t_{11} = 7.650$; $P < 0.001$; REMD/SWSD: $t_{11} = 8.900$; $P < 0.001$
REM in min	81.6	(7.1)	12.1*	(2.0)	63.5	(5.2)	undisturbed/REMD: $t_{11} = 10.909$; $P < 0.001$; REMD/SWSD: $t_{11} = -9.261$; $P < 0.001$
TST in min	403.4	(52.2)	342.7	(22.5)	337.1	(31.3)	
Delta power in μV^2	1008.3	(473.9)	1017.7	(479.1)	512.2*	(122.9)	undisturbed/SWSD: $t_{11} = 4.135$; $P = 0.002$; REMD/SWSD: $t_{11} = 4.355$; $P = 0.001$
SpD in 1/30s	4.00	(1.45)	3.91	(1.40)	3.95	(1.40)	
aSpA in mVs	24.24	(13.79)	22.50	(12.99)	24.43	(12.82)	
Forced awakenings	0	(0)	19.83	(7.81)	22.17	(8.45)	
Stress	2.8	(1.5)	4.5	(1.5)	4.8	(2.1)	
SSSe	2.50	(0.80)	1.92	(0.79)	1.67	(0.65)	
SSSm	3.00	(1.13)	3.08	(1.00)	3.33	(0.89)	
SSSrt	1.83	(0.72)	1.58	(0.67)	1.58	(0.51)	
mcons	129 %	(22)	120%	(20)	126%	(12)	
dcons	97.1 %	(12)	89.2%	(16)	94.1%	(9)	

Abbreviations: mean (M), standard deviation (SD), slow wave sleep (SWS), total sleep time (TST), spindle density in the whole night (SpD), absolute spindle activity in the whole night (aSpA), Stanford Sleepiness Scale (SSS) evenings (e), mornings (m) and at retest (rt), sleep dependent motor memory consolidation (mcons), sleep dependent declarative memory consolidation (dcons), REM sleep deprivation condition (REMD), slow wave sleep deprivation condition (SWSD)
*Significant difference to other experimental conditions

showed a significant difference between experimental conditions ($F_{14,32} = 2.62$; $P = 0.012$). The univariate *F*-test revealed a significant difference between the experimental conditions for delta power only ($F_{2,1} = 14.427$; $P < 0.001$). Using contrasts, the SWSD condition had significantly less delta power than both other experimental conditions (undisturbed/SWSD: $t_{11} = 4.135$; $P = 0.002$; REMD/SWSD: $t_{11} = 4.355$; $P = 0.001$). SWSD cut the delta power by half compared to both other conditions. The MANOVA for the spindle measures showed no significant difference between experimental conditions ($F_{8,60} = 0.502$; $P = 0.85$). Table 1 displays the stage 2 absolute spindle activity and spindle density in the whole night.

Learning Tasks

The MANOVA for the alertness data found no difference between the 3 experimental conditions for stress, sleepiness (SSS in the evening, morning, or at retest), and concentration (D2 in the evening, morning, or at retest) ($F_{14,54} = 1.468$; $P = 0.156$) (Table 1). The MANOVA for learning data revealed no difference between experimental conditions for the first tapping performance at training, tapping performance at the end of training, tapping performance at retest, motor memory consolidation (mcons), and declarative consolidation (dcons) ($F_{10,58} = 0.429$; $P = 0.927$).

The performance at the end of training phase of the finger tapping task was for the undisturbed condition 17.53 ± 4.18 seq/trial, the REMD condition 17.67 ± 2.96 seq/trial, and the SWSD condition 17.86 ± 3.59 seq/trial. At retest they were 22.14 ± 5.21 seq/trial, 21.06 ± 3.72 seq/trial, and 22.42 ± 4.74 seq/trial, respectively (Table 2 and Figure 1). Paired *t*-tests showed a significant difference between end-training performance and retest performance for all 3 experimental conditions (undisturbed: $t_{11} = -4.414$; $P < 0.001$; REMD: $t_{11} = -3.542$; $P = 0.003$; SWSD: $t_{11} = -6.899$; $P < 0.001$), which demonstrates an overnight enhancement in all 3 conditions. The overnight enhancement (mcons) was 129% ± 22% (undisturbed night), 120% ± 20% (REMD), and 126% ± 12% (SWSD) (Table 1). We found no correlations between mcons and absolute spindle activity (aSpA) and between mcons and spindle density (SpD) in the undisturbed night for stage 2 or NREM spindles (stage 2 spindles: aSpA: $r = 0.06$; $P = 0.427$; SpD: $r = 0.069$; $P = 0.416$; NREM spindles: aSpA: $r = -0.084$; $P = 0.397$; SpD: $r = -0.02$; $P = 0.475$), the REMD for stage 2 or NREM spindles (stage 2 spindles: aSpA: $r = 0.1$; $P = 0.378$; SpD: $r = 0.127$; $P = 0.348$; NREM spindles: aSpA: $r = 0.143$; $P = 0.328$; SpD: $r = 0.125$; $P = 0.35$), or SWSD condition for stage 2 or NREM spindles (stage 2 spindles: aSpA: $r = 0.02$; $P = 0.476$; SpD: $r = 0.136$; $P = 0.337$; NREM spindles: aSpA: $r = -0.003$; $P = 0.496$; SpD: $r = 0.109$; $P = 0.368$).

The average number of correct word pairs was 16.50 ± 2.11 after the undisturbed night, 15.17 ± 2.69 after REMD, and 16.00 ± 1.54 after SWSD; sleep dependent consolidation (dcons) was 97.1% ± 12%, 89.2% ± 16%, and 94.1% ± 9%, respectively (Table 1 and Figure 2).

Since we found no effect of the experimental conditions on learning data, and the experimental conditions differed in the amounts of SWS, REM sleep, and delta power, but not in the spindle measures, we performed a bivariate Pearson correlation between dcons and the spindle measures (aSpA and SpD). In the undisturbed condition we found a significant correlation between dcons and aSpA (stage 2 spindles: $r = 0.616$; $P = 0.017$;

Table 2— Mean Number and Standard Deviation of Correct Tapping Sequences

	End-training M (SD)	Retest M (SD)	T-test between end-training and retest
Undisturbed	17.53 (4.18)	22.14 (5.21)	$t_{11} = -4.414$; P < 0.001
REMD	17.67 (2.96)	21.06 (3.72)	$t_{11} = -3.542$; P = 0.003
SWSD	17.86 (3.59)	22.42 (4.74)	$t_{11} = -6.899$; P < 0.001

Mean number (M) and standard deviation (SD) of correct tapping sequences at the end of training in the evening and retest 60 hours later for the different experimental conditions: REM sleep deprivation condition (REMD), slow wave sleep deprivation condition (SWSD), and an undisturbed night. In all 3 conditions, significant enhancement in motor performance was found.

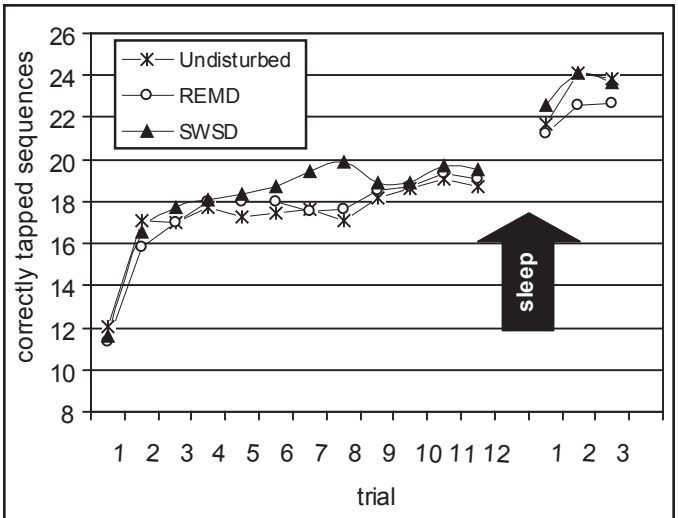

Figure 1—Performance at the finger tapping task before and after an undisturbed night, REM sleep deprivation (REMD), and slow wave sleep deprivation (SWSD) shown as mean number of correct tapped sequences for each trial of 30 sec. No significant difference was found between the conditions.

NREM spindles: r = 0.597; P = 0.02) (Figure 3) and between dcons and SpD (stage 2 spindles: r = 0.627; P = 0.015; NREM spindles: r = 0.58; P = 0.024). A closer look at the different parts of the night revealed an even more significant correlation between dcons and aSpA of the stage 2 spindles in the first third of the night (r = 0.794; P = 0.001), as well as between dcons and SpD of the stage 2 spindles in the first third of the night (r = 0.724; P = 0.004). The correlations to the other thirds of the night were for the second (aSpA: r = 0.529; P = 0.038; SpD: r = 0.574; P = 0.026) and the third (aSpA: r = 0.408; P = 0.094; SpD: r = 0.643; P = 0.012). The correlation between aSpA and dcons and between SpD and dcons in the REMD condition did not reach significance (stage 2 spindles: aSpA: r = 0.413; P = 0.091; SpD: r = 0.393; P = 0.103; NREM spindles: aSpA: r = 0.390; P = 0.105; SpD: r = 0.415; P = 0.09), and no correlation was found in the SWSD condition (stage 2 spindles: aSpA: r = 0.055; P = 0.432; SpD: r = 0.037; P = 0.455; NREM spindles: aSpA: r = 0.116; P = 0.36; SpD: r = 0.121; P = 0.354).

DISCUSSION

Surprisingly, we did not find that a severe decrease in the total amount of REM sleep or SWS affected the performance in learning tasks of young, healthy volunteers. In this experiment, we reduced REM sleep in the REMD condition and SWS in the SWSD condition by 85% and 75%, respectively, compared to the undisturbed condition. As in other studies,[17,30,45-47] the other sleep stages were also slightly but not significantly affected. In the SWSD condition, REM sleep was reduced by 22%, while in the REMD condition, SWS was reduced by 14%. The total sleep time was also diminished in both deprivation conditions. In all conditions sleepiness, concentration, and stress were the same. Nevertheless, in all 3 conditions (REMD, SWSD, and undisturbed), sleep-dependent consolidation of the motor and declarative tasks was attained. We did, however, find a significant correlation between declarative memory consolidation and the spindle measures—absolute spindle activity and spindle density—in sleep stage 2 and NREM in the undisturbed condition, especially in the first third of the night. The subjects spent 2 recovery nights at home before returning for the retest. We chose this interval period to eliminate effects of the deprivation protocol on concentration and sleepiness at retest. This was possible since it has been shown that only the first night after learning is significant for sleep-dependent consolidation and cannot be compensated by the following nights.[25] In this section, we begin by discussing possible explanations for the lack of influence of REMD/SWSD on motor and declarative tasks and then go on to discuss the dependence of declarative memory on sleep spindles.

REMD/SWSD and Motor Tasks

Two recent studies have utilized REMD and procedural memory tasks.[47,48] Neither study found an effect of REMD on test performance. There are 2 possible explanations for REMD and SWSD not influencing sleep-dependent consolidation of motor tasks: (1) the diminished amount of REM sleep in the REMD condition was still sufficient for sleep-dependent memory consolidation of the relatively easy task used for this study, or (2) the memory consolidation is dependent on stage 2 sleep, rather than on REM sleep or SWS.

Recent studies of short midday naps have indicated that very small amounts of sleep have resulted in motor skill enhancement.[41,49,50] This may imply that the diminished amount of REM sleep in the REMD condition in our study was still enough to consolidate the motor task. Additional evidence for this explanation comes from animal studies, which have shown that REM sleep-dependent memory processing may be based on ponto-geniculo-occipital (PGO) waves, known to appear just prior to the onset of and throughout REM sleep.[51,52] In these experiments, the activation of a PGO wave generator prevented memory impairment induced by REMD.[52] Therefore, if PGO waves occurred in our study just prior to awakenings, their presence may have been sufficient to consolidate the motor task despite the REMD condition.

On the other hand, it has recently been proposed that although REM sleep is involved with the reprocessing of procedural tasks that require a new cognitive strategy, simple and familiar motor tasks involve primarily stage 2 sleep.[38] This theory convincingly

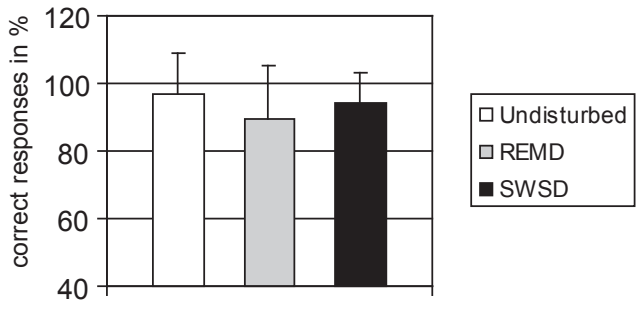

Figure 2—Percentile of word pairs recalled (dcons) in regard to the learning baseline at retest 60 hours after an undisturbed night, REM sleep deprivation (REMD), and slow wave sleep deprivation (SWSD).

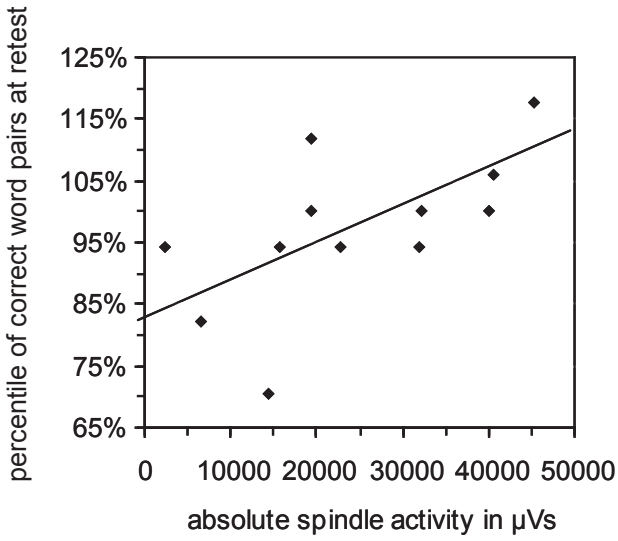

Figure 3—Relationship between spindle activity and declarative memory performance in the undisturbed condition. Shown is a significant correlation (P = 0.017) between stage 2 absolute spindle activity and the percentile of correct word pairs at retest in regard to the learning baseline.

explains many discrepancies seen in tests of procedural and motor learning tasks, since until recently the "newness" or "complexity" of the learning tasks had been neglected. For example, experiments using a complex motor task (mirror-tracing) have reported an increase in subsequent REM sleep and a vulnerability to REM deprivation.[9,53] This is in contrast to studies of simple motor tasks (e.g., rotary pursuit, finger tapping), which have shown such tasks to correlate with the amount of stage 2 sleep and to be adversely affected by stage 2 sleep disruptions and not REMD.[2,18,47] Recently, further correlations have been found between the overnight enhancement of simple motor tasks and stage 2 spindle density, as well as an increase of stage 2 spindle density after motor learning.[38,39,41-43] We explore the evidence for this effect in our study at the end of this section.

REMD/SWSD and Declarative Tasks

As with motor tasks, there are 2 possible explanations for the lack of influence of REMD/SWSD on declarative tasks: (1) the diminished amount of SWS in the SWSD condition was still adequate for declarative memory consolidation, or (2) the declarative task relies on other sleep characteristics, such as sleep spindles.

It has recently been shown in studies utilizing daytime naps and the verbal paired associates task (PAL) that short naps can generate both an improvement in number of word pairs recalled and a percentage improvement over baseline comparable to that resulting from a whole night of sleep.[54,55] This demonstrates that approximately 22 min of SWS, the mean amount from these experiments, is probably sufficient for the memory consolidation of small declarative tasks. Since, for our study, the mean of amount of SWS in the SWSD condition was 26.5 min, this may have provided ample time for the consolidation of our declarative task. However, the link between small amounts of SWS and declarative memory remains tenuous and not well understood; while it has been shown that a nap with as little as 6 min of stage 1 and 2 sleep could consolidate declarative tasks,[19] it has also been found that a mean amount of 7.1 min of SWS did not result in an improvement of declarative memory (PAL).[49] Further, a correlation between SWS in the first 2 hours of a whole night and improved performance in a non-declarative has also been shown.[56] Since it seems impossible to totally deprive SWS in a whole night, further "nap" studies evaluating the minimum amount of SWS necessary for declarative memory consolidation would be interesting. Until now only one study using the acoustic stimulation technique for SWSD achieved almost complete deprivation.[57] But a recent study using the same technique had a rest-SWS amount of 26 min, which is the same amount as we had.[58]

Given the lack of effect in our study between the amounts of SWS, REM sleep, and delta power on learning tasks, we then analyzed the spindle measures, which were unchanged in all our conditions and which are often associated with memory consolidation.[35-44] In the undisturbed condition, we found a significant correlation between the declarative task and the spindle measures in stage 2 and NREM: absolute spindle activity and spindle density. This correlation is particularly strong when considering only the absolute spindle activity and spindle density in stage 2 in the first third of the night. Previously, Plihal and Born reported the importance of the first half of the night for declarative memory consolidation.[9] Since SWS is the most dominant sleep stage during this interval, they deduced that the declarative task relied on SWS. Our correlation findings may indicate, however, that the importance of the first half of the night for declarative memory consolidation originates from sleep spindles and not SWS. Likewise, the connection between spindle activity and memory consolidation has been increasingly seen in the past few years, with correlations mainly being found with stage 2 spindles, and not stage 3 spindles. With regard to declarative memory tasks, the density of stage 2 spindles has been shown to increase after extensive learning of these tasks, and the degree of increase in the stage 2 spindle activity correlates with memory performance.[35-37,40,44,59] These results speak for the importance of stage 2 sleep with its sleep spindles for declarative learning.

It has frequently been speculated that spindles may be important for synaptic plasticity.[60] In one study, a correlation was found between the retention of memories and the spindles de-

tected over parietal regions, the region in which the "fast" spindles are located.[59] Further "fast" spindles were associated with the overnight improvement of memory,[34,35,61] while "slow" spindles only increased after learning in highly gifted individuals.[35] Regrettably, we did not have the technical abilities to perform an analysis of fast and slow spindles. In-vitro artificial spindle-like stimulation has also been shown to induce short-term and long-term potentiation in neocortical pyramid cells.[62] This has been explained by Sejnowski and Destexhe as a 2-step process by which spindle oscillations open "molecular gates to plasticity" through a massive Ca^{2+} influx, and then proceed in SWS by iteratively "recalling" and "storing" information primed in neural assemblies.[63] Evidence for this hypothesis has been found in studies of artificially induced slow oscillation-like potential fields, which have been shown to enhance declarative memory.[64] In the present study, we halved the delta power representing the natural slow oscillations of sleep in our SWSD condition, while we decreased SWS by 75%. Our results may therefore indicate that the delta power is the more important measure than SWS in general in sleep dependent memory consolidation.

Caveats

While our results presented here may indicate that sleep spindles and not REM sleep/SWS are the driving force in memory consolidation, our experiment was not designed to test this effect, so some ambiguities remain. In particular, sleep spindles have been linked to general cognitive and learning abilities,[34,53,65,66] and spindle activity is generally elevated in highly gifted students.[35] These results could indicated that the correlations we found do not reflect a connection between sleep dependent memory enhancement and sleep spindle activity, but rather that the subjects with high spindle activity are generally better learners. Since we did not attempt to explicitly test sleep spindles, we did not include a "non-learning" baseline night and therefore cannot reliably distinguish between these 2 effects.

We also note that the correlation between sleep spindles and declarative consolidation found here is primarily in the undisturbed condition for stage 2 and NREM spindles. In marked contrast to the strong connection between declarative memory consolidation and sleep spindles in the undisturbed condition, this correlation in the REMD condition did not reach significance. Furthermore, in the SWSD condition, no correlation was found at all. This should be regarded as a caveat that sleep deprivation may have had a subtle effect on consolidation processes that was not seen in the behavior. These results are nevertheless difficult to interpret in the context of sleep spindles as an indicator of memory consolidation.

A further caveat for our results is our relatively small number of subjects. This is a general shortcoming in sleep research. We tried to minimize negative effects by using a within-subject design. Despite this caveat, we feel that the present data are sufficiently strong to exclude large effects of the intervention on memory consolidation.

Implications for the Decline in Learning Abilities with Age

Learning abilities in general, and sleep dependent memory consolidation specifically, are known to decline with age.[43,67-70] It has been suggested that this decline is related to the degeneration of sleep architecture and the decrease of SWS and REM sleep in the elderly.[67,69,71] On average, the amount of SWS during a normal night sleep in healthy subjects is reduced from approximately 77 min in 20-year-olds to approximately 38 min in 70-year-olds, while the amount of REM sleep decreases from approximately 86 min to approximately 57 min in the respective age groups.[72] Sleep quality in the elderly is further diminished by increasing number of awakenings, as well as varying degrees of spontaneous disruptions of the NREM/REM cycle.[69,71]

In this study, the various deprivation conditions resulted in a similar but stronger disturbance of the subjects' sleep, with no resulting inhibition of learning. This implies that diminishing sleep-dependent consolidation in the elderly is not solely associated with reduced amounts of SWS/REM sleep or increased numbers of awakenings and spontaneous disruptions of the NREM/REM cycle. Other factors that change with age, such as hormones and neurotransmitters, must therefore play an important role.

The aging process is associated with decreased levels of most hormones, increased levels of cortisol in the first half of the night, and changes in cholinergic neurotransmission.[69,73] For example, the cognition of healthy older adults has been improved by growth hormone releasing hormone.[74] In this scenario, memory augmentation in elderly subjects induced by pharmacological cholinergic manipulation[75] may not have been achieved indirectly by the REM sleep enhancing effect of the drug as assumed, but rather through the direct influence of the neurotransmitter. This would also explain why REM sleep augmentation reached by REM sleep rebound does not affect sleep-dependent learning.[48]

In conclusion, it seems that sleep-dependent memory consolidation does not rely only on intact amounts of SWS or REM sleep across a night of sleep. The process more likely requires different EEG microstructures, for example sleep spindles, delta waves, and PGO waves. Exploring the dependence of memory consolidation on these other factors may be an important avenue for future research.

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REFERENCES

1. Gais S, Born J. Declarative memory consolidation: mechanisms acting during human sleep. Learn Mem 2004;11:679-85.
2. Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R. Practice with sleep makes perfect: sleep-dependent motor skill learning. Neuron 2002;35:205-11.

3. Ellenbogen JM. The sleeping brain's influence on memory. Sleep 2008;31:163-4.
4. Smith C. Sleep states and memory processes in humans: procedural versus declarative memory systems. Sleep Med Rev 2001;5:491-506.
5. Rauchs G, Desgranges B, Foret J, Eustache F. The relationship between memory systems and sleep stages. J Sleep Res 2005;14:123-40.
6. Stickgold R, Walker MP. Memory consolidation and reconsolidation: what is the role of sleep. Trends Neurosci 2005;28:408-15.
7. Stickgold R. Sleep-dependent memory consolidation. Nature 2005;437:1272-8.
8. Fanjaud G, Clavet U, De Feneyrols R, Barrere M, Bes A, Arbus L. Role du sommeil paradoxal dans l'apprentissage chez l'homme. Rev EEG Neurophysiol 1982;12:337-43.
9. Plihal W, Born J. Effects of early and late nocturnal sleep on declarative and procedural memory. J Cognitive Neurosci 1997;9:534-47.
10. Barrett T, Ekstrand B. Effect of sleep on memory. III: Controlling for time of day effects. J Exp Psychiatry 1972;96:321-7.
11. Fowler M, Sullivan M, Ekstrand B. Sleep and memory. Science 1973;179:302-304.
12. Yaroush R, Sullivan M, Ekstrand B. Effect of sleep on memory. II: Differential effect of first and second half of the night. J Exp Psychiatry 1971;88:361-366.
13. Empson JAC, Clarke P. Rapid eye movements and remembering. Nature 1970;227:287-8.
14. O'Keefe J, Nadel L. The hippocampus as a cognitive map. New York: Oxford University Press, 1978.
15. Tilley AJ, Empson JAC. REM sleep and memory consolidation. Biol Psychol 1978;6:293-300.
16. Walker MP, Stickgold R. Sleep, memory, and plasticity. Annu Rev Psychol 2005;12.1-12.28.
17. Karni A, Tanne D, Rubenstein BS, Askenasy JJM, Sagi D. Dependence on REM Sleep of overnight improvement of perceptual skill. Science 1994;265:679-82.
18. Smith C, MacNeill C. Impaired motor memory for a pursuit rotor task following Stage 2 sleep loss in college students. J Sleep Res 1994;3:206-13.
19. Lahl O, Wispel C, Willigens B, Pietrowsky R. An ultra short episode of sleep is sufficient to promote declarative memory performance. J Sleep Res 2008;17:3-10.
20. Tucker AM, Dinges DF, Van Dongen HPA. Trait interindividual differences in the sleep physiology of healthy young adults. J Sleep Res 2007;16:170-80.
21. Van Dongen HPA, Baynard MD, Maislin G, Dinges DF. Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. Sleep 2004;27:423-33.
22. Brickenkamp R. Test d2, Aufmerksamkeits-Belastungs-Test. 9 ed. Göttingen, Bern, Toronto, Seattle: Hogrefe, 2002.
23. Hoddes E, Zarcone V, Smythe H, Phillips R, Dement W. Quantification of sleepiness: a new approach. Psychophysiology 1973;10:431-6.
24. Brown RM, Robertson EM. Off-line processing: reciprocal interactions between declarative and procedural memories. J Neurosci 2007;27:10468-75.
25. Stickgold R, James L, Hobson JA. Visual discrimination learning requires sleep after training. Nat Neurosci 2000;3:1237-8.
26. Baker FC, Mitchell D, Driver HS. Oral contraceptives alter sleep and raise body temperature in young women. Eur J Physiol 2001;424:729-37.
27. Baker FC, Driver HS. Circadian rhythms, sleep, and menstrual cycle. Sleep Med 2007;8:613-622.
28. Wright KP Jr, Badia P. Effects of menstrual cycle phase and oral contraceptives on alertness, cognitive performance, and circadian rhythms during sleep deprivation. Behav Brain Res 1999;103:185-94.
29. Fischer S, Hallschmid M, Elsner AL, Born J. Sleep forms memory for finger skills. Proc Natl Acad Sci U S A 2002;99:11987-91.
30. Endo T, Roth C, Landolt H-P, et al. Selective REM sleep deprivation in humans: effects on sleep and sleep EEG. Am J Physiol Regul Integr Comp Physiol 1998;274:1186-94.
31. Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Bethesda, MD: U.S. Department of Health, Education and Welfare, Public Health Services, 1968.
32. Grözinger M, Beersma DGM, Fell J, Röschke J. Is the nonREM-REM sleep cycle reset by forced awakenings from REM sleep? Physiol Behav 2002;77:341-7.
33. Anderer P, Gruber G, Parapatics S, et al. An E-Health solution for automatic sleep classification according to Rechtschaffen and Kales: Validation study of the Somnolyzer 24 x 7 utilizing the siesta database. Neuropsychobiology 2005;51:115-33.
34. Schabus M, Hödlmoser K, Gruber G, et al. Sleep spindle-related activity in the human EEG and its relation to general cognitive and learning abilities. Eur J Neurosci 2006;23:1738-46.
35. 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.
36. Schabus M, Gruber G, Parapatics S, et al. Sleep spindles and their significance for declarative memory consolidation. Sleep 2004;27:1479-85.
37. Clemens Z, Fabó D, Halász P. Overnight verbal memory retention correlates with the number of sleep spindles. Neuroscience 2005;132:529-35.
38. Fogel SM, Smith C. Learning-dependent changes in sleep spindles and stage 2 sleep. J Sleep Res 2006;15:250-5.
39. Fogel SM, Smith C, Cote KA. Dissociable learning-dependent changes in REM and non-REM sleep in declarative and procedural memory systems. Behav Brain Res 2007;180:48-61.
40. Gais S, Mölle M, Helms K, Born J. Learning-dependent increases in sleep spindle density. J Neurosci 2002;22:6830-4.
41. Nishida M, Walker MP. Daytime naps, motor memory consolidation and regionally specific sleep spindles. PLoS ONE 2007;2:e341.
42. Peters KR, Smith V, Smith C. Changes in sleep architecture following motor learning depend on initial skill level. J Cognitive Neurosci 2007;19:817-29.
43. Peters KR, Ray L, Smith V, Smith C. Changes in the density of stage 2 spindles following motor learning in young and older adults. J Sleep Res 2008;17:33.
44. 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.
45. Grözinger M, Kögel P, Röschke J. Effects of REM sleep awakenings and related wakening paradigms on the ultradian sleep cycle and the symptom in depression. J Psychiatr Res 2002;36:299-308.
46. Hornung OP, Regen F, Schredl M, Heuser I, Danker-Hopfe H. Manipulating REM sleep in older adults by selective REM sleep deprivation and physiological as well as pharmacological REM sleep augmentation methods. Exp Neurol 2006;197:486-94.
47. Saxvig IW, Lundervold AJ, Gronli J, Ursin R, Bjorvatn B, Portas CM. The effect of a REM sleep deprivation procedure on different aspects of memory function in humans. Psychophysiology 2008;45:309-17.
48. Hornung OP, Regen F, Danker-Hopfe H, Schredl M, Heuser I. The relationship between REM sleep and memory consolidation in old age and effects of cholinergic medication. Biol Psychiatry 2007;61:750-7.

49. Backhaus J, Junghanns K. Daytime naps improve procedural motor memory. Sleep Med 2006;7:508-12.
50. Korman M, Doyon J, Doljansky J, Carrier J, Dagan Y, Karni A. Daytime sleep condenses the time course of motor memory consolidation. Nat Neurosci 2007;10:1206-13.
51. Callaway CW, Lydic R, Baghdoyan HA, Hobson JA. Pontogeniculooccipital waves: spontaneous visual system activity during rapid eye movement sleep. Cell Mol Neurobiol 1987;7:105-49.
52. Datta S. Activation of phasic pontine-wave generator: a mechanism for sleep-dependent memory processing. Sleep Biol Rhythms 2006;4:16-26.
53. Smith C, Nixon MR, Nader R. Posttraining increases in REM sleep intensity implicate REM sleep in memory processing and provide a biological marker of learning potential. Learn Mem 2004;11:714-719.
54. Tucker MA, Fishbein W. Enhancement of declarative memory performance following a daytime nap is contingent on strength of initial task acquisition. Sleep 2008;31:197-203.
55. Tucker MA, Hirota Y, Wamsley EJ, Lau H, Chaklader A, Fishbein W. A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. Neurobiol Learn Mem 2006;86:241-7.
56. Huber R, Ghilardi MF, Massimini M, Tononi G. Local sleep and learning. Nature 2004;430:78-81.
57. Ferrara M, De Gennaro L, Curcio G, Cristiani R, Corvasce C, Bertini M. Regional Differences of the human sleep electroencephalogram in response to selective slow-wave sleep deprivation. Cereb Cortex 2002;12:737-48.
58. Aeschbach D, Cutler AJ, Ronda JM. A role for non-rapid-eye-movement sleep homeostasis in perceptual learning. J Neurosci 2008;28:2766-72.
59. Clemens Z, Fabó D, Halász P. Twenty-four hours retention of visuospatial memory correlates with the number of parietal sleep spindles. Neurosci Lett 2006;403:52-56.
60. Steriade M. Coherent oscillations and short-term plasticity in corticothalamic networks. Trends Neurosci 1999;22:337-45.
61. Milner CE, Fogel SM, Cote KA. Habitual napping moderates motor performance improvements following a short daytime nap. Biol Psychology 2006;73:141-56.
62. Rosanova M, Ulrich D. Pattern-specific associative long-term potentiation induced by a sleep spindle-related spike train. J Neurosci 2005;25:9398-405.
63. Sejnowski TJ, Destexhe A. Why do we sleep? Brain Res 2000;886:208-23.
64. Marshall L, Helgadóttir H, Mölle M, Born J. Boosting slow oscillations during sleep potentiates memory. Nature 2006;444:610-613.
65. Fogel SM, Nader R, Cote KA, Smith C. Sleep spindles and learning potential. Behav Neurosci 2007;121:1-10.
66. Bodizs R, Kis T, Lazar AS, et al. Prediction of general mental ability based on neural oscillation measures of sleep. J Sleep Res 2005;14:285-92.
67. Buckley TM, Schatzberg AF. Aging and the role of the HPA axis and rhythm in sleep and memory-consolidation. Am J Geriatr Psychiatry 2005;13:344-52.
68. Dresler M, Kluge M, Genzel L, Schüssler P, Steiger A. Synergistic effects of age and depression on sleep-dependent memory consolidation. Pharmacopsychiatry 2007;40:238-9.
69. Hornung OP, Danker-Hopfe H, Heuser I. Age-related changes in sleep and memory: commonalities and interrelationships. Exp Gerontol 2005;40:279-85.
70. Spencer RMC, Gouw AM, Ivry RB. Age-related decline of sleep-dependent consolidation. Learn Mem 2007;14:480-4.
71. Mazzoni G, Gori S, Formicola G, et al. Word recall corrrelates with sleep cycles in elderly subjects. J Sleep Res 1999;8:185-8.
72. Danker-Hopfe H, Schäfer M, Dorn H, et al. Percentile reference charts for selected sleep parameters for 20- to 80-year old healthy subjects from the SIESTA database. Somnologie 2005;9:3-14.
73. Steiger A. Sleep and the hypothalamo-pituitary-adrenocortical system. Sleep Med Rev 2002;6:125-38.
74. Vitiello MV, Moe KE, Merriam GR, Mazzoni G, Buchner DH, Schwartz RS. Growth hormone releasing hormone improves the cognition of healthy older adults. Neurobiol Aging 2006;27:318-23.
75. Schredl M, Weber B, Leins M-L, Heuser I. Donepezil-induced REM sleep augmentation enhances memory performance in elderly, healthy persons. Exp Gerontol 2001;36:353-61.