The Relationship Between REM Sleep and Memory Consolidation in Old Age and Effects of Cholinergic Medication

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Background: Recent findings in young adults suggest that rapid eye movement (REM) sleep plays a role in procedural memory consolidation. The significance of REM sleep for memory consolidation in old age has not yet been investigated.

Methods: Effects of REM sleep manipulation on declarative and procedural memory consolidation were investigated in 107 healthy older adults, ages 60–82 years. Rapid eye movement sleep deprivation was achieved by REM sleep awakenings and compared with non-REM sleep awakenings. Rapid eye movement sleep augmentation was realized physiologically by REM sleep rebound and pharmacologically by administering an acetylcholinesterase inhibitor in a double-blind, placebo-controlled design. Memory performance was tested by a paired associate list and a mirror tracing task at 9:30 PM and 7:30 AM with sleep intervening between 11:00 PM and 7:00 AM.

Results: Although REM sleep deprivation led to a significant reduction in total and phasic REM sleep, memory consolidation remained unaffected. Both REM sleep augmentation groups showed a significant increase in phasic REM sleep, whereas only pharmacological cholinergic REM sleep manipulation exerted a significant positive effect on procedural memory consolidation.

Conclusions: Because only after cholinergic stimulation of phasic REM sleep procedural memory consolidation is improved, cholinergic activation seems to be a crucial component of REM sleep-related memory consolidation in old age.

Key Words: Acetylcholinesterase inhibitor, age-related memory decline, aging, Alzheimer's disease, memory consolidation, REM sleep

The relationship between rapid eye movement (REM) sleep and memory consolidation has been investigated for more than 3 decades. Several of the early studies demonstrated that selective REM sleep deprivation leads to significant impairments in overnight memory retention (Empson and Clarke 1970; Tilley and Empson 1978). However, other early studies failed to find such effects, raising serious doubts about the actual importance of REM sleep in the consolidation of newly formed memory traces (Chernik 1972; Ekstrand et al 1971). Selective REM sleep deprivation requires the interruption of REM sleep by awakenings or arousals, whenever REM onset is identified during the course of sleep, and is usually compared with equal disturbance of non-REM (NREM) sleep (Hornung et al 2006).

More recently, a possible explanation for the inconsistency among findings with regard to REM sleep and memory consolidation has been elaborated by distinguishing between different memory systems, such as declarative and procedural memory (Smith 2001). Declarative memory (i.e., memory for facts and events) critically depends on the integrity of the hippocampus, whereas procedural memory (i.e., memory for skills and habits) relies primarily on the striatum (Squire and Zola 1996). Previous research indicates that declarative memory benefits from early nocturnal sleep, when slow wave sleep (SWS) predominates, whereas procedural memory is enhanced through late nocturnal sleep, when REM sleep prevails (Plihal and Born 1997; Plihal et al 1999). Similarly, selective REM sleep deprivation was found to impair procedural memory consolidation compared with selective deprivation of SWS (Karni et al 1994). Other findings suggest that an optimal level of memory consolidation is only reached if SWS precedes REM sleep during the course of sleep (Gais et al 2000; Stickgold et al 2000).

The significance of REM sleep for memory consolidation has also been implied by findings regarding the effects of an intensive cognitive training during wakefulness on subsequent REM sleep. In several past studies, an enhancement of REM sleep duration or phasic (i.e., eye movement-related) REM sleep activity has been observed after various forms of cognitive training during wakefulness (Buchegger et al 1991; Smith and Lapp 1991; Verschoor and Holdstock 1984). Moreover, a significant correlation between learning efficiency and increases in REM sleep percentage was found in students taking part in a foreign language course (De Koninck et al 1989). These findings indicate that REM sleep plays an important role in plasticity-related processes. Likewise, functional neuroimaging data have revealed that brain areas involved in performing a cognitive task during wakefulness might be reactivated during subsequent REM sleep, a process that seems to be modulated by prior acquisition levels (Laureys et al 2001; Maquet et al 2000; Peigneux et al 2003). In rats, posttraining increases in REM sleep as well as phasic pontine-wave activity have been observed (Datta 2000; Datta et al 2005). Interestingly, the memory impairing effects of REM sleep deprivation in rats can be prevented by an activation of the phasic pontine-wave generator during posttraining sleep (Datta et al 2004). Therefore, not only REM sleep duration but also phasic components of REM sleep should be considered in the context of REM sleep-related memory consolidation.

It is well known that aging affects both REM sleep as well as memory-related processes (Hornung et al 2005). With regard to REM sleep, age-related changes such as decreased REM sleep percentage, shortened REM latency, as well as reduced REM density have been observed (Carrier et al 1997; Danker-Hopfe et

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al 2005; Darchia et al 2003). Furthermore, power decreases in electroencephalographic frequencies below 10 Hz have been reported for REM sleep in older adults compared with younger ones (Landolt and Borbély 2001; Landolt et al 1996). Age-related changes in declarative memory are reflected by a decline in recall and recognition of new events and facts with increasing age (Prull et al 2000). With regard to procedural memory, older adults seem to benefit less from breaks in between learning sessions compared with younger adults, suggesting age-related deficits in latent learning processes (Raz et al 2000; Wright and Payne 1985). At present, it is unclear whether the observed changes in REM sleep and memory processes with increasing age are interrelated.

According to previous studies, REM sleep could play an important role in cognitive functioning of older adults. In this context, REM sleep percentage was found to be positively associated with overnight memory improvement in healthy older adults after the acetylcholinesterase inhibitor (AChE-I) donepezil had been administered before bedtime for 6 consecutive nights (Schredl et al 2001). Similarly, a positive association between increased REM sleep percentage and cognitive improvement was reported for Alzheimer's patients over a 6-week course of donepezil administration (Mizuno et al 2004). Moreover, a positive association between REM sleep duration and longitudinal measures of memory performance across a preceding time interval of 18 years was observed in older adults (Prinz 1977). However, despite the interesting implications, the significance of REM sleep for memory consolidation in old age has not yet been investigated systematically (Hornung et al 2005).

In the present study, the significance of REM sleep for memory consolidation was investigated within a sample of healthy older adults. Total and phasic REM sleep were differentiated in this context, because phasic components of REM sleep seem to be of particular importance for memory consolidation during REM sleep (Datta et al 2004). On the basis of previous research in younger adults, it was hypothesized that different amounts of total and phasic REM sleep duration would affect procedural but not declarative memory consolidation in older adults (Karni et al 1994; Plihal and Born 1997; Plihal et al 1999). More specifically, it was expected that selective REM sleep deprivation would lead to a significant impairment in overnight procedural memory consolidation compared with stage 2 NREM sleep awakenings of equal frequency. Physiological rebound-based and pharmacological cholinergic REM sleep augmentation were both anticipated to improve overnight procedural memory consolidation compared with a placebo condition. In accordance with these assumptions, the durations of total and phasic REM sleep were hypothesized to be positively associated with morning procedural memory performance in the study sample. In younger adults, REM sleep enhancement has been observed after various forms of cognitive training, and posttraining REM sleep activity seems to be related to learning efficiency and acquisition levels (De Koninck et al 1989; Peigneux et al 2003; Verschoor and Holdstock 1984). For exploratory purposes, the association between evening levels of memory performance and subsequent total and phasic REM sleep duration was investigated in the placebo group of the present study.

Methods and Materials

Participants

Participants were recruited by advertisements placed in local newspapers. Altogether, 120 healthy older adults, male and female, between the ages of 60 and 85 were included in the study. To rule out any significant medical impairments, an initial screening regarding medical history and medication use was conducted in a structured telephone interview. An extensive medical screening procedure followed, consisting of a detailed medical interview; physical, psychiatric, and neurological examinations; electroencephalogram (EEG); electrocardiogram (ECG); routine blood chemistry; and urine analysis. To screen for mild cognitive impairment, a Mini-Mental State Examination (MMSE) and a Clock-Drawing Test were performed. A minimum score of 29 was required in the MMSE and a perfect clock had to be produced in the Clock-Drawing Test for inclusion in the study. None of the participants reported subjective memory complaints or a progressive memory decline over time. A structured interview and a 100-item questionnaire regarding sleep habits and history of sleep disturbances were applied to assess sleep quality in the participants. Usual bedtimes between 10:00 PM and 12:00 midnight, usual sleep durations of at least 6 hours, and a history without sleep disorders were required for participation. Two weeks before the study participants were asked to fill out a 14-day sleep diary to ensure a regular sleep-wake rhythm. Furthermore, participants were instructed not to nap before the nights in the sleep laboratory. Use of medication known to affect sleep or memory-related processes was excluded. The present study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the ethics committee of the Charité–University Medicine Berlin, Campus Benjamin Franklin. All participants gave written informed consent and were paid for study participation.

Screened participants were randomly assigned to five experimental groups of REM sleep manipulation with a sample size of 24/group. Before the study all participants spent one adaptation night in the sleep laboratory, on the basis of which 13 participants were excluded from further analyses with reference to the following sleep-related inclusion criteria: apnea index $<$ 15/hour, blood oxygen saturation $>$ 90%, periodic leg movements (PLMS), arousal index $<$ 20/hour, total arousal index $<$ 33.7/hour, and absence of any other specific sleep disorder. Of the remaining 107 participants, 51.4% were female and the age range was between 60 and 82 years with a mean age of 66.1 years ($\pm$ 5.1 SD). According to the results from the Morningness-Eveningness Questionnaire (MEQ (Horne and Östberg 1976)), 67.9% of the participants were classified as morning type, 31.0% as intermediate type, and 1.2% as evening type with respect to their circadian rhythm. The five experimental groups did not differ significantly with regard to age, gender, and MEQ score, as illustrated in Table 1.

Study Design

The adaptation night was immediately followed by the subsequent study protocol, where the research question was approached from two different angles (i.e., REM sleep deprivation and REM sleep augmentation). Selective REM sleep deprivation was achieved by repeated awakenings at the first clear signs of REM sleep indicated by the occurrence of muscle atonia together with rapid eye movements and REM sleep-specific EEG activity. To control for the adverse effects of the awakenings as such, a second group was woken during stage 2 NREM sleep in matched frequency (selective REM sleep deprivation group n = 24; stage 2 NREM sleep awakening group n = 20). Owing to the well known differences in distribution patterns of sleep stages across nighttime sleep, the time course of the awakenings could not be matched. To allow for SWS and REM sleep with stage 2 NREM

Table 1. Demographic Data and Results From the Morningness-Eveningness Questionnaire in the Five Experimental Groups

	REMD $n = 24$	NREMA $n = 20$	Rebound $n = 21$	AChE-I $n = 22$	Placebo $n = 20$	Statistics
Age, Years (mean ± SD)	67.6 ± 6.1	66.0 ± 4.9	65.8 ± 5.0	65.5 ± 4.8	65.4 ± 4.6	$F(4,102) = .728$, ns
Gender						
Female (%)	45.8	45.0	52.4	63.6	50.0	χ^2 (4) = 1.968, ns
Male (%)	54.2	55.0	47.6	36.4	50.0	
Morningness-Eveningness						
Morning type (%)	72.2	70.6	61.5	66.7	66.7	χ^2 (4) = .477, ns[a]
Intermediate type (%)	22.2	29.4	38.5	33.3	33.3	
Evening type (%)	5.6	0	0	0	0	

REMD, selective rapid eye movement (REM) sleep deprivation; NREMA, stage 2 non-REM (NREM) sleep awakenings. Rebound, physiological rebound-based REM sleep augmentation; AChE-I, pharmacological cholinergic REM sleep augmentation; Placebo, placebo group.

[a]Morningness-eveningness was categorized into "morning type" and "non-morning type" (intermediate or evening type) for statistical testing.

sleep awakenings, the experimental awakenings began after the first NREM-REM cycle in this group. All participants were kept awake for 5 min after each experimental awakening by filling out visual analogue scales concerning their subjective well-being. This was done to prevent a fast relapse into REM sleep after the REM sleep awakenings. The experimental awakenings were carried out by experienced polysomnography scorers.

The REM sleep augmentation was realized either physiologically through REM sleep rebound or pharmacologically by the AChE-I donepezil. Physiological rebound-based REM sleep augmentation relied on selective REM sleep deprivation the night preceding the study night and, therefore, required 3 consecutive nights in the sleep laboratory (rebound group $n = 21$). Daytime activity between the selective REM sleep deprivation night and the study night was supervised to prevent any napping to interfere with REM sleep rebound during the study night. Pharmacological cholinergic REM sleep augmentation was achieved by administering 5 mg of the AChE-I donepezil orally to participants 30 min before bedtime at 10:30 PM in a double-blind, placebo-controlled design (medication group $n = 22$; placebo group $n = 20$). Donepezil is an AChE-I approved for the symptomatic treatment of mild to moderate Alzheimer's disease, which reaches peak plasma concentrations about 3–4 hours after oral administration and has an elimination half-life of about 70 hours.

To investigate the significance of REM sleep for memory consolidation in old age, all participants took part in declarative and procedural memory tasks before and after the study night. The memory tasks were carried out at 9:30 PM the evening before the study night and at 7:30 AM the morning thereafter. An overview of the study design is displayed in Figure 1.

Sleep Recording

Polysomnographic recordings took place from 11:00 PM until 7:00 AM throughout the nights in the sleep laboratory. Sleep EEG from central (C3 and C4) and occipital (Oz) derivation points was recorded in all nights along with electromyogram (EMG) mentalis and submentalis, electrooculogram (EOG) horizontal and vertical, as well as blood oxygen saturation. During the adaptation night, nasal airflow, snoring, thoracic and abdominal excursion, as well as EMG tibialis left and right were additionally registered to screen for breathing-related sleep disorders, such as central or obstructive sleep apnea, as well as PLMS. The 264 polysomnographically recorded nights were visually scored on a 30-sec basis according to the rules published by Rechtschaffen and Kales (1968). Sleep EEG was rated by seven experienced scorers who had received extensive prior training. One of these scorers controlled the ratings of all nights, and consensus scoring was applied in case of controversial epochs.

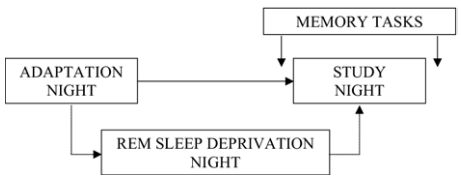

Figure 1. Study design. After the adaptation night, participants took part in the subsequent study the following night. An additional night of selective rapid eye movement (REM) sleep deprivation preceded the study night only in the physiological rebound-based REM sleep augmentation group. Before and after the study night, declarative and procedural memory tasks were performed by all of the participants.

Measures of REM Sleep

The durations of total and phasic REM sleep in minutes were chosen as measures of REM sleep during the study night. Total REM sleep duration in minutes refers to the amount of REM sleep across the whole study night. The duration of phasic REM sleep in minutes was calculated on the basis of total REM sleep duration and REM density. The REM density was defined as the ratio of 3-sec epochs during REM sleep accompanied by rapid eye movements to all 3-sec epochs during REM sleep. Phasic REM sleep duration was calculated according to the formula [total REM sleep duration × REM density].

Memory Tasks

Evening and morning declarative memory performance was tested by a paired associate word list. The evening before the study night, a list of 34 paired associates was read out to the participants at a rate of 3 sec / word pair. Thereafter, participants were asked to recall the target words of the paired associate list whenever the corresponding cue words were read out, and direct feedback was given with regard to the correctness of the answers. Afterward the list of paired associates was read out again and a second recall trial followed. The morning after the study night, a third recall trial was conducted in the same fashion and after the list of paired associates had been read out again a fourth recall trial followed. To rule out primacy and recency effects, the first and last two paired associates of the list remained the same across the trials, serving as buffer items, which were not included in the statistical analyses. The remaining 30 paired associates were presented in randomized order in each trial to further exclude serial position effects. For statistical analyses, the number of errors (i.e., words not recalled) was averaged within evening as well as morning trials, resulting in one evening and one morning mean error score.

Evening and morning procedural memory performance was tested by a mirror tracing task. In this task, a light-sensitive stylus was used to draw along black lines of geometric figures, which could only be viewed through a mirror. The evening before the study night, a simple practice figure in the shape of a house was drawn seven times to acquire a basic level of the mirror tracing skill. Thereafter, six more complex test figures that consisted of three adjacent squares arranged in horizontal or diagonal patterns were presented consecutively. The outer lines of the test figures were 1 cm wide, 58 cm long, and contained 12 right angles. The morning after the study night, participants were asked to draw the practice figure and each of the six test figures one more time. Procedural memory performance was measured by recording number of errors (i.e., number of offline instances), performance time, and error time (i.e., time offline) for each test figure. For statistical analyses, average scores of performance measures within the evening and morning test figures were calculated, resulting in one evening and one morning mean score for each of the three performance measures.

Only complete sets of evening and morning testings were included in the statistical analyses of each specific performance measure. Therefore, the sample sizes of the experimental groups varied between $n = 19$ and $n = 24$ in the declarative memory task and between $n = 16$ and $n = 21$ in the procedural memory task. The distribution of the procedural memory task data showed a substantial positive skewness, which was accounted for by logarithmic transformation. According to the Kolmogorov-Smirnov test, evening declarative memory task data and log transformed procedural memory task data were normally distributed.

Statistical Analysis

Statistical analyses were conducted with SPSS 13.0 for Windows (SPSS, Chicago, Illinois). General linear model (GLM) univariate and repeated measures analyses were applied to investigate experimental group differences in a between subjects design with regard to measures of sleep and in a within subjects design with regard to evening and morning memory performance measures. Selective REM sleep deprivation was compared with stage 2 NREM sleep awakenings, whereas physiological rebound-based and pharmacological cholinergic REM sleep augmentation groups were compared with the placebo condition. Pearson's product moment correlation was applied to investigate the association between REM sleep and memory performance. It was replaced by partial correlation when further variables needed to be controlled for. Missing data were treated by listwise deletion in each specific analysis, and the significance level was set to $p < .05$ (two-tailed).

Results

Experimental Manipulation of REM Sleep

During the study night, selective REM sleep deprivation led to a significant reduction in total and phasic REM sleep duration compared with stage 2 NREM sleep awakenings [respectively, $F(1,42) = 27.809$, $p < .001$; $F(1,42) = 42.817$, $p < .001$]. When REM sleep augmentation and placebo groups were compared, a significant overall group effect was found with regard to phasic REM sleep duration [$F(2,60) = 3.248$, $p < .05$] but not total REM sleep duration [$F(2,60) = 2.663$, ns]. Physiological rebound-based as well as pharmacological cholinergic REM sleep augmentation approaches both led to a significant increase in phasic REM sleep duration compared with the placebo group [respectively, $F(1,39) = 5.148$, $p < .05$; $F(1,40) = 6.263$, $p < .05$]. The results of the experimental manipulation of REM sleep are illustrated in Figure 2.

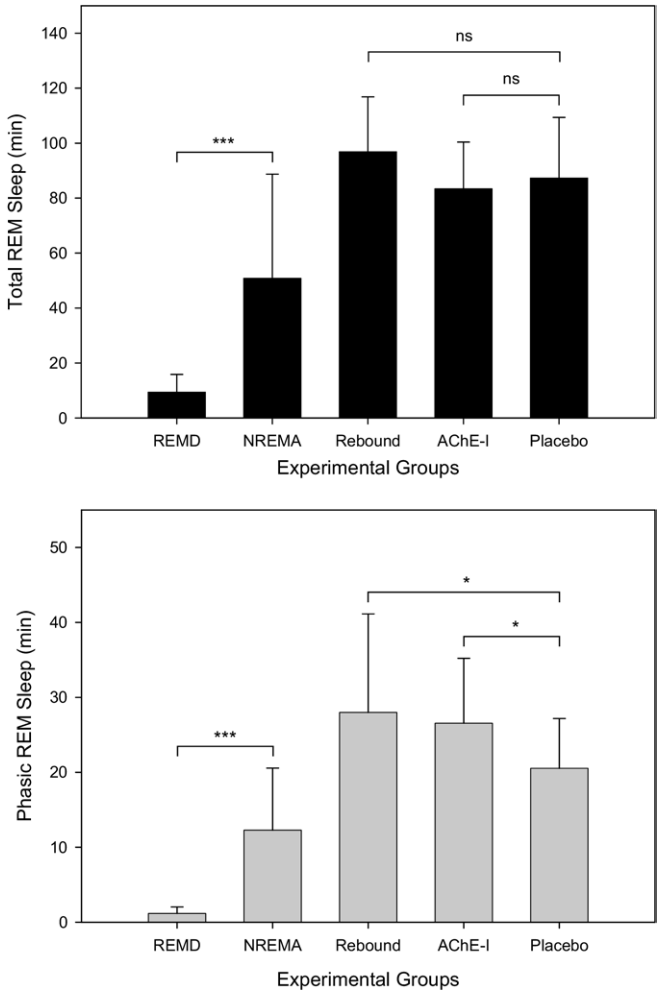

Figure 2. Experimental manipulation of rapid eye movement (REM) sleep. Displayed are means and SDs of total (black bars) and phasic (grey bars) REM sleep duration during the study night in the five experimental groups. $^{*}p < .05$; $^{***}p < .001$. Abbreviations as in Table 1.

Additional information with regard to group differences in sleep period time, wake time, duration of NREM sleep stages, and REM density during the study night is supplied in Table 2.

Effects on Declarative and Procedural Memory Consolidation

Table 3 displays evening and morning performance measures of the declarative and procedural memory tasks for each of the five experimental groups. It is of interest to note that the five experimental groups did not differ significantly with regard to any of the evening memory performance measures. Moreover, significant overnight improvements were observed for all measures of memory performance in each of the five experimental groups.

In a repeated measures design, overnight performance improvement in the declarative and procedural memory tasks was compared between the experimental groups, with evening and morning memory performance measures entered as within subjects variables and factor group as between subjects variable. When comparing selective REM sleep deprivation with stage 2

Table 2. Sleep Period Time, Wake Time, Duration of non-REM (NREM) Sleep Stages and Rapid Eye Movement (REM) Density (Mean ± SD) During the Study Night in the Five Experimental Groups

	REMD $n = 24$	NREMA $n = 20$	REMD vs. NREMA $F(1,42)$	Rebound $n = 21$	AChE-I $n = 22$	Placebo $n = 20$	Rebound vs. Placebo $F(1,39)$	AChE-I vs. Placebo $F(1,40)$
Sleep Period Time (min)	450.3 ± 28.8	452.3 ± 22.9	.065	454.2 ± 24.4	446.6 ± 26.4	449.3 ± 19.5	.499	.141
Wake Time (min)	161.0 ± 55.5	159.2 ± 70.3	.009	27.4 ± 15.3	57.1 ± 35.8	51.3 ± 32.8	9.121[a]	.292
Stage 1 NREM (min)	86.2 ± 43.0	78.5 ± 30.8	.446	58.2 ± 29.1	90.7 ± 46.3	65.1 ± 32.1	.519	4.235[b]
Stage 2 NREM (min)	167.8 ± 64.3	144.3 ± 54.5	1.665	230.0 ± 37.0	187.9 ± 34.3	210.8 ± 53.7	1.795	2.778
Slow Wave Sleep (min)	31.6 ± 30.1	25.2 ± 26.8	.548	45.9 ± 34.1	31.7 ± 31.4	40.1 ± 36.1	.283	.647
REM density (%)	18.8 ± 16.7	25.3 ± 14.8	1.846	28.4 ± 11.9	32.0 ± 9.2	24.5 ± 8.8	1.448	7.207[b]

Abbreviations as in Table 1.
[a] $p < .01$.
[b] $p < .05$.

NREM sleep awakenings, no significant time × group interaction was found with regard to overnight declarative memory improvement in number of errors [$F(1,42) = .376$, ns] or overnight procedural memory improvement in log number of errors, log performance time, and log error time [respectively, $F(1,38) = .809$, ns; $F(1,38) = .002$, ns; $F(1,38) = .001$, ns]. Similarly, REM sleep augmentation and placebo groups did not show any significant time × group interaction with regard to overnight performance improvement in number of errors of the declarative memory task [$F(2,59) = .414$, ns]. However, a significant time × group effect emerged with regard to overnight performance improvement in log number of errors and log error time of the procedural memory task [respectively, $F(2,49) = 3.264$, $p < .05$; $F(2,49) = 5.588$, $p < .01$]. In this context, only the pharmacological cholinergic REM sleep augmentation approach led to a significant increase in overnight performance improvement compared with the placebo group, as illustrated in Figure 3. With regard to overnight performance improvement in log performance time of the procedural memory task, no significant time × group interaction was observed between REM sleep augmentation and placebo groups [$F(2,50) = .431$, ns]. Finally, it is of interest to note that the two awakening groups did not show any significant differences in overnight memory performance improvement compared with the placebo group.

REM Sleep and Morning Memory Performance

To further specify the relationship between REM sleep and memory consolidation in the present study, the association of total and phasic REM sleep duration with morning declarative and procedural memory performance was investigated across all groups, while statistically controlling for the effects of cholinergic medication by partial correlational analysis. In this context, no significant associations were observed between total and phasic REM sleep duration and morning declarative memory performance regarding number of errors [respectively, $r(103) = -.008$, ns; $r(103) = .171$, ns]. In contrast, total and phasic REM sleep duration proved to be significantly associated with morning log number of errors in the procedural memory task [respectively, $r(99) = -.226$, $p < .05$; $r(99) = -.221$, $p < .05$]. No significant associations were found between total and phasic REM sleep duration and morning procedural log performance time [respectively, $r(99) = -.020$, ns; $r(99) = -.050$, ns] or log error time [respectively, $r(99) = -.140$, ns; $r(99) = -.159$, ns]. It is important to note that the associations between total and phasic REM sleep duration and morning log number of errors in the procedural memory task failed to reach significance when evening memory performance levels were also statistically controlled for in the partial correlational analyses.

Evening Memory Performance and REM Sleep

For exploratory purposes, the association between evening levels of memory performance and subsequent total and phasic REM sleep duration was investigated. These analyses were conducted in the placebo group, where sleep architecture of the study night had remained unaffected by experimental awakenings, REM sleep rebound, or cholinergic medication. In this

Table 3. Evening and Morning Memory Performance Measures (mean ± SD) in the Five Experimental Groups

	REMD	NREMA	Rebound	AChE-I	Placebo	Statistics
Declarative Memory Task						
Number of errors	$n = 24$	$n = 20$	$n = 21$	$n = 22$	$n = 19$	
Evening	12.8 ± 3.5	13.3 ± 3.3	13.5 ± 5.2	14.5 ± 4.0	12.3 ± 3.6	$F(4,101) = .915$,ns
Morning	6.9 ± 3.0[a]	7.0 ± 2.9[a]	7.6 ± 5.1[a]	8.2 ± 3.5[a]	6.7 ± 3.3[a]	
Procedural memory task						
Log number of errors	$n = 21$	$n = 19$	$n = 18$	$n = 17$	$n = 17$	
Evening	1.3 ± .5	1.3 ± .4	1.1 ± .5	1.4 ± .6	1.3 ± .4	$F(4,87) = 1.033$,ns
Morning	1.1 ± .5[a]	.9 ± .4[a]	.9 ± .4[b]	1.0 ± .5[a]	1.0 ± .3[b]	
Log Performance Time	$n = 21$	$n = 19$	$n = 18$	$n = 18$	$n = 17$	
Evening	1.9 ± .2	1.8 ± .2	1.9 ± .1	2.0 ± .2	1.9 ± .1	$F(4,88) = 1.331$,ns
Morning	1.8 ± .2[a]	1.7 ± .2[b]	1.8 ± .2[a]	1.8 ± .2[a]	1.8 ± .2[a]	
Log Error Time	$n = 21$	$n = 19$	$n = 18$	$n = 18$	$n = 16$	
Evening	1.0 ± .4	.9 ± .4	.9 ± .4	1.1 ± .4	1.0 ± .3	$F(4,87) = .826$,ns
Morning	.8 ± .4[a]	.7 ± .3[b]	.7 ± .4[b]	.7 ± .4[a]	.8 ± .4[a]	

Abbreviations as in Table 1.
[a] $p < .001$.
[b] $p < .01$ tested in a repeated measures analysis of evening and morning memory performance measures.

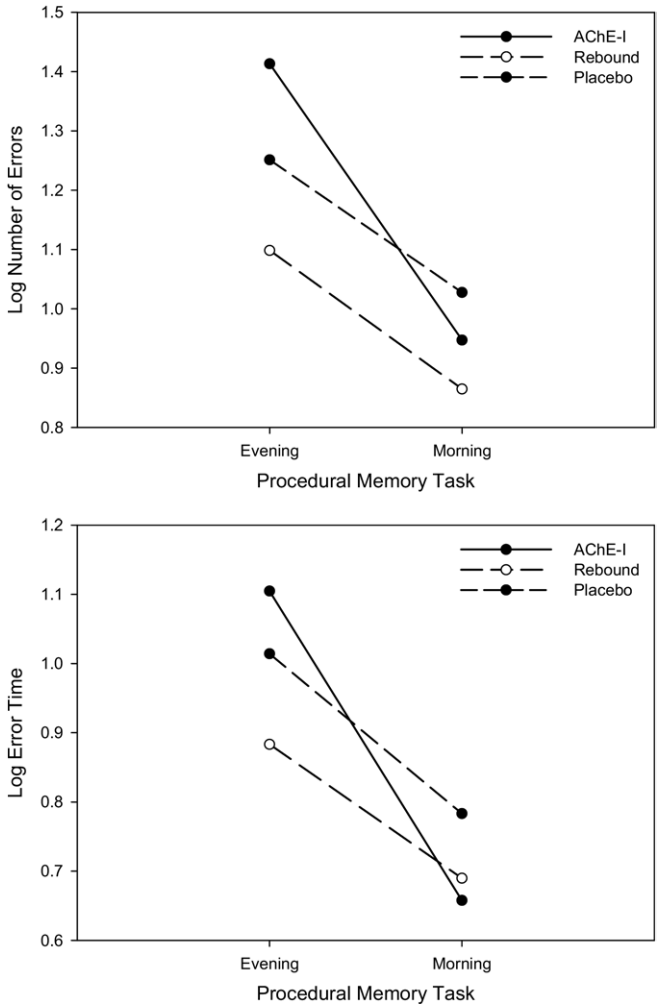

Figure 3. Effects of rapid eye movement (REM) sleep manipulation on procedural memory consolidation. Displayed are mean evening and morning performance measures (at the top: log number of errors; at the bottom: log error time) in the REM sleep augmentation and placebo groups. When comparing the pharmacological cholinergic REM sleep augmentation approach to the placebo condition, a significant time × group interaction was found with regard to log number of errors as well as log error time [respectively, $F(1,32) = 5.659$, $p < .05$; $F(1,32) = 8.239$, $p < .01$]. In contrast, no significant time × group effect was observed for the physiological rebound-based REM sleep augmentation approach in this context [respectively, $F(1,33) = .009$, ns; $F(1,32) = .197$, ns]. Abbreviations as in Table 1.

context, evening number of errors in the declarative memory task did not show any significant association with subsequent total or phasic REM sleep duration the following night [respectively, $r(18) = -.112$, ns; $r(18) = -.029$, ns]. With regard to evening procedural memory performance, a significant association was found between evening log performance time and subsequent total REM sleep duration [$r(15) = -.570$, $p < .05$] but not phasic REM sleep duration [$r(15) = -.133$, ns]. In contrast, evening log error time in the procedural memory task was significantly associated with subsequent phasic REM sleep duration [$r(14) = .579$, $p < .05$] but not total REM sleep duration [$r(14) = .114$, ns]. Evening procedural memory performance did not prove to be significantly associated with total and phasic REM sleep duration in respect of log number of errors [respectively, $r(15) = .135$, ns; $r(15) = .265$, ns]. The significant associations between evening procedural memory performance and REM sleep are illustrated in Figure 4.

Discussion

Previous research in young adults suggests that REM sleep plays a role in procedural memory consolidation (Karni et al 1994; Plihal and Born 1997; Plihal et al 1999). In contrast to this, the findings of the present study demonstrate that even with significant group differences regarding total and phasic REM sleep duration, no significant effects on overnight procedural memory consolidation are found in older adults. This indicates that REM sleep does not critically affect procedural memory consolidation in old age, which is further supported by the weak association between REM sleep and morning procedural memory performance in the present study. Only in association with cholinergic medication did we find a significant improvement in overnight procedural memory consolidation, indicating that cho-

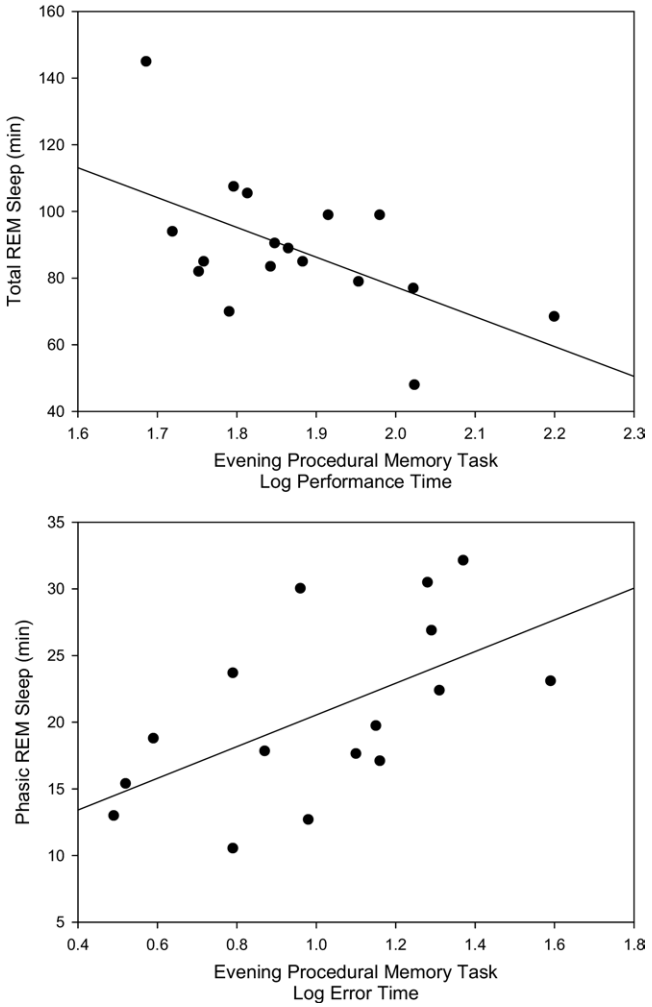

Figure 4. Association between evening levels of procedural memory performance and subsequent total and phasic rapid eye movement (REM) sleep duration. At the top: shorter log evening performance times were associated with longer durations of total REM sleep the following night [$r(15) = -.570$, $p < .05$]. At the bottom: higher log evening error times were associated with longer durations of subsequent phasic REM sleep duration [$r(14) = .579$, $p < .05$].

linergic activation might in fact be a crucial component for REM sleep-related memory consolidation in old age.

There is substantial evidence that stage 2 NREM sleep and SWS also contribute to sleep-related memory consolidation (Stickgold 2005). In the present study, the pharmacological cholinergic REM sleep augmentation group and the placebo condition did not differ significantly with regard to stage 2 NREM sleep and SWS, as illustrated in Table 2. Hence, the beneficial effects of the pharmacological cholinergic REM sleep augmentation approach regarding overnight procedural memory consolidation were not based on the manipulation of these sleep stages. It might be argued that the improvement in overnight procedural memory consolidation with cholinergic medication was based solely on memory enhancing effects of the drug at the time of retrieval testing, independent of any sleep-related processes (Freo et al 2005). Because the AChE-I donepezil has an elimination half-life of about 70 hours, morning procedural memory performance could have been significantly promoted by the medication. However, such a medication-based enhancement should have also affected declarative memory, which was not the case in the present study. According to a recent study in rats, intact acetylcholine neurotransmission seems to be a prerequisite for memory consolidation during REM sleep (Legault et al 2004). Therefore, REM sleep-related memory consolidation in older adults could be impaired owing to age-related deficits in cholinergic neurotransmission, which are counteracted by cholinergic medication (Terry and Buccafusco 2003).

It is of interest to note that whereas both REM sleep augmentation groups showed a significant increase in phasic REM sleep duration, only the pharmacological cholinergic REM sleep augmentation approach led to a significant increase in REM density (Table 2). Previous research suggests that the memory-impairing effects of REM sleep deprivation in rats can be prevented by an activation of the phasic pontine-wave generator, which is closely related to REM activity (Datta et al 2004). Present findings suggest that the intensity of phasic activity during REM sleep, as indicated by REM density, is of higher relevance for plasticity-related processes than the duration of it. A recent study in young adults showed that procedural memory consolidation is not promoted by cholinergic activation during early nocturnal sleep, which is rich in SWS but contains only little REM sleep (Gais and Born 2004). Therefore, a certain amount of REM sleep seems to be necessary to promote procedural memory consolidation through cholinergic activation during sleep. In the same study, declarative memory consolidation during early nocturnal sleep was found to be impaired by cholinergic activation. Hence, declarative memory consolidation could have also been impaired in the pharmacological cholinergic REM sleep augmentation group of the present study. We did not find such an effect; however, medication-based enhancement of memory performance at the time of retrieval testing might have compensated in part for this.

In young adults, REM sleep enhancement has been observed after various forms of cognitive training, and posttraining REM sleep activity seems to be related to learning efficiency and acquisition levels (De Koninck et al 1989; Peigneux et al 2003; Verschoor and Holdstock 1984). In the present study, differential findings were obtained with regard to the relationship between evening levels of procedural memory performance and subsequent total and phasic REM sleep duration. Whereas a negative association was found between performance time and total REM sleep duration, a positive correlation was observed between error time and phasic REM sleep duration. On the basis of the present study design, it cannot be determined whether evening levels of procedural memory performance and subsequent REM sleep characteristics are causally linked or whether they just both represent a stable personality trait. Nevertheless, this is an interesting finding that should be further investigated in a study where REM sleep data from the study night is compared with a baseline night not preceded by training.

This study has several limitations. Sleep deprivation paradigms have been criticized repeatedly for unspecific side effects of the awakening protocols, such as stress and fatigue (Horne 2000; Siegel 2001; Vertes 2004). The two REM sleep augmentation paradigms were introduced to circumvent these problems; however, other critical aspects need to be considered in this context. In the physiological rebound-based REM sleep augmentation group, evening memory encoding could have been impaired by the preceding night of REM sleep deprivation, whereas in the pharmacological cholinergic REM sleep augmentation group, morning retrieval testing might have been promoted by the medication, independent of any sleep-related processes. Moreover, even though the mirror tracing task has been applied repeatedly in the context of sleep and learning (Gais and Born 2004; Plihal and Born 1997), other frequently used tasks such as finger sequence tapping or visual texture discrimination might be more sensitive to sleep effects in older adults (Karni et al 1994; Manoach et al 2004). Of course, age-related changes in circadian rhythm and memory performance also need to be considered. Older adults are inclined to sleep earlier in the evening and to wake up earlier in the morning compared with younger adults (Carrier et al 1997; Phillips and Ancoli-Israel 2001). In the present study, evening testings took place at 9:30 PM, which might have been too late for optimal encoding in older adults. Furthermore, age-related increases in interindividual as well as intraindividual variability have been observed for a variety of cognitive measures, including memory performance (Li et al 2001). This increased variability might have obscured the potential effects of REM sleep manipulation on memory consolidation in the present study.

In conclusion, because only after cholinergic stimulation of phasic REM sleep procedural memory consolidation is improved, cholinergic activation seems to be a crucial component of REM sleep-related memory consolidation in old age. To our knowledge, this is the first study to investigate the significance of REM sleep for declarative and procedural memory consolidation in older adults. Future research should examine the qualitative components of REM sleep contributing to plasticity-related processes in old age in further detail. Understanding the significance of REM sleep for memory consolidation in healthy older adults might also help to advance our knowledge of mechanisms in Alzheimer's disease, where declining memory function is associated with reduced REM sleep and changes in cholinergic neurotransmission.

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