Differential effect of an anticholinergic antidepressant on sleep-dependent memory consolidation

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Abstract

Background

Rapid eye movement (REM) sleep is considered critical to the consolidation of procedural memory – the memory of skills and habits. Many antidepressants strongly suppress REM sleep, however, and procedural memory consolidation has been shown to be impaired in depressed patients on antidepressant therapy. As a result, it is important to determine whether antidepressive therapy can lead to amnestic impairment.

Methods

We thus investigated the effects of the anticholinergic antidepressant amitriptyline on sleep-dependent memory consolidation in 25 healthy men (mean age: 26.8 ± 5.6 years) in a double-blind, placebo-controlled, randomized parallel-group study.

Results

Our findings show that amitriptyline profoundly suppressed REM sleep \( t(23) = -8.02, P < 0.001 \) and impaired perceptual skill learning \( F(1, 23) = 4.99, P = 0.04 \), but not motor skill or declarative learning.

Conclusions

Our study is the first to demonstrate that an antidepressant can affect procedural memory consolidation in healthy subjects. Moreover, considering the results of a recent study, in which selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors were shown not to impair procedural memory consolidation, our findings suggest that procedural memory consolidation is not facilitated by the characteristics of REM sleep captured by visual sleep scoring, but rather by the high cholinergic tone associated with REM sleep. Our study contributes to the understanding of the behavioral toxicity of antidepressants, which are among the most widely prescribed drugs worldwide.
Introduction

Numerous studies have shown that there is a close association between sleep and memory consolidation (1-3). Rapid eye movement (REM) sleep is thought to play a role in the memory consolidation process, particularly with regard to procedural memory – the memory of skills and habits (4-6). Many antidepressants, however, strongly suppress REM sleep (7), and procedural memory consolidation has been shown to be impaired in depressed patients who are undergoing antidepressant therapy (8, 9). In light of these findings, it seems reasonable to ask whether antidepressive therapy can lead to amnestic impairment, especially considering that antidepressants have become the most commonly prescribed class of medications in the United States (10).

To our knowledge, only one study to date has investigated the effects of antidepressants on memory consolidation during sleep in healthy young subjects (11). Its results showed that suppressing REM sleep with the selective serotonin reuptake inhibitor (SSRI) fluvoxamine or the serotonin-norepinephrine reuptake inhibitor (SNRI) reboxetine did not impair procedural memory consolidation. When interpreting this finding it is important to consider that SSRIs and SNRIs suppress only those characteristics of REM sleep that are captured by visual sleep stage scoring; while both classes of compounds allow the high cholinergic brain activity naturally associated with REM sleep to persist. However, the alternation between high levels of acetylcholine during wakefulness and REM sleep and low levels of acetylcholine during slow-wave sleep (SWS) are thought to be critical to the memory consolidation process (12-14).

In contrast to SSRIs and SNRIs, anticholinergic antidepressants suppress both REM sleep and high cholinergic brain activity, and may thus affect memory consolidation. Indeed, procedural memory consolidation was shown to be impaired in subjects whose cholinergic receptors were blocked using the muscarinic receptor antagonist scopolamine and the
nicotinic receptor antagonist mecamylamine during late nocturnal sleep, which is rich in REM sleep (15). By the same token, enhancing cholinergic tone by administering the acetylcholine esterase inhibitor donepezil improved procedural memory performance in healthy older adults (16).

We thus investigated the effects of the anticholinergic antidepressant amitriptyline in a double-blind, placebo-controlled, parallel-group study of 32 healthy young subjects. The subjects performed two procedural memory tasks (a visual discrimination task measuring perceptual skill learning, and a finger tapping task measuring motor skill learning) and two declarative memory tasks (Rey-Osterrieth Complex Figure Test, Rey Auditory-Verbal Learning Test). After the training session, subjects received either amitriptyline or placebo and spent the night in a sleep lab for polysomnographic recording. Retrieval testing took place 24 hours after the training session (Figure 1). Whereas improvement on the visual discrimination task is known to depend on post-training REM sleep (4, 5), performance gains on the other tasks performed in our study are thought to be linked to stage 2 sleep or SWS only (17, 18). We hypothesized that the amitriptyline and placebo groups would differ in their performance on the visual discrimination task but not on the other tasks.

**Methods and Materials**

**Subjects**

Subjects were recruited through bulletin board announcements and a subject recruitment registry maintained by the Institute of Psychology at Humboldt University in Berlin, Germany. Inclusion criteria were (a) male gender, (b) age 18 through 40 years, and (c) ability to communicate effectively in German. Exclusion criteria were (a) shift work within the past 24 months, (b) any sleep disorder as measured by the Pittsburgh Sleep Quality Index (19), (c) irregular sleep/wake patterns or extreme chronotype as measured by the Morningness-Eveningness Questionnaire (20, 21), (d) history of any neurologic or psychiatric disorders, (e)
regular medication intake within the past four weeks, (f) contraindications for amitriptyline, or (g) an abnormal electrocardiogram (ECG).

Approximately 500 men were screened by telephone interview, but most were excluded due to irregular sleep-wake patterns or extreme chronotype. Ultimately, a total of 25 healthy subjects aged 18 through 39 years (mean age: 26.8 ± 5.6 years) with normal or corrected-to-normal vision were included in the final analysis. The study protocol was approved by the local ethics committee and the German Federal Institute for Drugs and Medical Devices (EudraCT 2007-003546-14). After complete description of the study to the subjects, written informed consent was obtained. Prior to the study, all subjects underwent physical and mental health examinations.

*Experimental design and procedure*

Subjects participated in the study for 11 days. During the first eight days, they maintained regular sleep schedules as confirmed by sleep logs and actigraphy (Actiwatch, Cambridge Neurotechnology). On the ninth day, subjects spent an adaptation night in the sleep lab to accustom them to sleeping under laboratory conditions. The next morning (day 10), subjects left the sleep lab and pursued their usual daily activities. They returned to the sleep lab that evening for the training session, which started at 6:00 pm and entailed performing two procedural memory tasks (a visual discrimination task measuring perceptual skill learning and a finger tapping task measuring motor skill learning) and two declarative memory tasks (the Rey-Osterrieth Complex Figure Test and the Rey Auditory-Verbal Learning Test). Subsequently, subjects were randomized in a double-blind manner to an amitriptyline group or a placebo group, receiving amitriptyline 25 mg at 9:30 pm and 50 mg at 1:30 am or placebo while remaining in bed between 11:00 pm and 7:00 am for polysomnographic recording. The next morning (day 11), subjects were required to fill out a morning protocol (22) by answering questions about their current physical and mental state, and about the restorative value of their sleep (i.e., on a five-point scale ranging from “very restorative” to
“not restorative at all”). After completing the morning protocol, subjects left the sleep lab and followed their daily activities. They were not allowed to nap until they had completed retrieval testing, which took place at 6:00 pm. Retrieval testing was conducted in the same manner as during the training session 24 hours earlier (Figure 1).

Memory tasks

In the visual discrimination task, introduced by Karni and Sagi to measure perceptual skill learning (23), the perception threshold was measured by identifying the orientation of a target texture. The task was presented on a standard personal computer with a 17-inch monitor (75 Hz) using Presentation® 12.1 software (Neurobehavioral Systems Inc.). Subjects reacted by pressing keys on a standard German QWERTZ keyboard. They were instructed to fixate the center of the screen throughout the trial. A centered cross was displayed first. Subjects began the first trial of the task by pressing the space bar, after which a sequence of screens was presented: a blank screen (300 ms), a stimulus pattern (10 ms; Figure 3A), a blank screen (stimulus-to-mask interval of 460–60 ms according to block number), a mask pattern to erase the retinal image of the stimulus (100 ms; Figure 3B), and a response screen (without time limit). Subjects had to answer whether the letter at the center of the stimulus pattern was a “T” or an “L”, and whether the diagonal bars were aligned horizontally or vertically. Immediate auditory feedback was given only for letter identification, which served as a fixation control task, and the only trials ultimately included in the analysis were those in which the letter had been identified correctly. Subjects’ ability to discriminate visual textures was assessed using the orientation of the bars. Visual discrimination difficulty was increased systematically by decreasing the stimulus-to-mask interval (stimulus onset asynchrony; SOA). All subjects completed 25 blocks of 50 trials, with one block each at SOAs of 460, 360, 260, and 220 ms, and three blocks each at SOAs of 180, 160, 140, 120, 100, 80, and 60 ms, leading to total of 1250 trials in the training session and 1250 trials in the retrieval testing session. Before the training session, subjects completed a block of 50 trials with an SOA of 460 ms in the presence of the investigator. Performance was measured as the
percentage of correct responses at each SOA. The perception threshold was estimated by interpolating the point at which the rate of correct responses was 80%. Improvement in this task was defined as a decrease in the perception threshold between training and retrieval testing.

In the finger tapping task (18), which tests motor skill learning, the five-element sequence 4-1-3-2-4 had to be tapped on a keyboard with the fingers of the non-dominant hand as quickly and as accurately as possible for a period of 30 seconds. Subjects performed this trial a total of 12 times with 30-second breaks between each trial. The numeric sequence was displayed on the screen to reduce working-memory demands, and each key press resulted in a white rectangle being displayed. Every trial was scored for speed (i.e., the number of correctly completed sequences) and accuracy (i.e., the number of errors made). The average scores for speed and accuracy on the last two trials were used as a measure of training performance. During retrieval testing, only two trials were performed, and average scores were generated. Changes in performance were calculated as differences in speed and accuracy between training and retrieval testing.

The Rey-Osterrieth Complex Figure Test is a widely used neuropsychological test for evaluating visual declarative memory (24). Subjects were given a card that showed a line drawing (i.e., the so-called stimulus figure) and asked to copy this drawing by hand using a piece of paper and a pencil. When they had finished, both the stimulus figure and their reproduction of it were removed, and without prior warning the subjects were asked to reproduce the figure again, but from memory. During retrieval testing, subjects had to draw the figure yet again from memory. Memory performance was scored using criteria related to location, accuracy, and organization. Changes in performance were calculated as differences in the scores obtained during training and retrieval testing.
The Rey Auditory-Verbal Learning Test is used to assess verbal declarative memory (24). The test consists of 15 unrelated nouns (list A) that are read aloud to the subject at a rate of one noun per second for five consecutive trials (trials 1–5). Each trial was followed by a free-recall test. After completing the fifth trial, subjects were presented with an interference list of 15 words (list B) and subsequently asked to reproduce these words in a free-recall test (trial 6). Finally, subjects were asked to recall list A again (trial 7). Retrieval testing consisted of a free-recall test of list A (i.e., without this list having been presented again in the meantime). The difference between the number of words remembered in the last training trial and during retrieval testing served as an indicator of a change in performance.

On average, subjects needed 90 to 120 minutes to complete all four memory tasks. The tasks were performed in a silent environment with one subject in each room. For the visual discrimination task, the room was darkened. All memory tasks were administered and evaluated by the same neuropsychologist (M.G.).

Polysomnographic recording and sleep data analysis
Sleep was polygraphically recorded for two consecutive nights using Sagura Polysomnograph 2000 (Dr. Sagura RMS AG). The recordings were performed using standard filter settings and included six electroencephalogram (EEG) channels (F3-A2, F4-A1, C3-A2, C4-A1, O1-A2, O2-A1), two electrooculogram (EOG) channels, a mental electromyograph (EMG) channel, an EMG channel for the tibialis anterior muscle of each leg, and electrocardiography (ECG). In addition, nasal air flow, thoracic and abdominal excursion, peripheral oxygen saturation, and rectal (core body) temperature were measured. Sleep was scored according to the standardized criteria of Rechtschaffen and Kales in 30-second epochs by two experienced scorers who were blind to the treatment and to the results of the memory tasks (25). For the time in bed (TIB; i.e., time from lights off to lights on), every epoch was scored as (a) wake, (b) non-REM sleep stage 1, 2, 3, or 4, or (c) REM sleep. Time spent in non-REM stages 3 and 4 was defined as slow-wave sleep (SWS). “Sleep
onset” was defined as the first epoch of stage 2 sleep; “end of sleep” as the first epoch of wake without a subsequent epoch of sleep; “sleep latency” as the time from lights off to sleep onset; “REM latency” as the time from sleep onset to the first epoch of REM sleep; “sleep period time” (SPT) as the time from sleep onset to the end of sleep; “total sleep time” (TST) as SPT minus wake after sleep onset; “percentage of a sleep stage” as the percentage of SPT; “sleep efficiency” as the ratio of TST to TIB; “awakenings” as the sum of periods with at least one epoch awake during SPT; “latency after awakening” as minutes needed to reach sleep stage 2, 3, 4, or REM sleep again after being awakened for medication administration; “wake without latency after awakening” as minutes spent awake during SPT minus latency after awakening.

Statistical analysis

Seven subjects were excluded from the analysis: one because of an abnormal electroencephalogram (epileptic potentials), three due to technical difficulties, and three because of poor sleep during the post-training night. Poor post-training sleep means that the subjects (a) slept less than 80% of their habitual sleep time as measured by sleep logs in the seven nights before the experiment and (b) had rated their sleep as being “not very restorative” or “not restorative at all” in the morning protocol after the post-training night. Data analysis was thus performed using datasets from 25 subjects, 12 of whom were in the amitriptyline group (mean age: 25.2 ± 3.6 years) and 13 of whom were in the placebo group (mean age: 28.3 ± 6.8 years). Due to right-handedness, data from an uneven number of subjects (11 amitriptyline, 13 placebo) were included in the analysis for the finger tapping task.

Data were analyzed using PASW Statistics 18 (SPSS Inc.). Variables were tested for normal distribution using the Shapiro-Wilk test. Comparative analyses were carried out using unpaired Student’s t tests where the data were normally distributed; otherwise, exact Mann-
Whitney U tests were performed. Interaction effects were tested using a mixed-design ANOVA. A two-tailed P value less than 0.05 was considered significant.

**Results**

In concordance with the findings of other studies, amitriptyline increased REM sleep latency \([t(23) = 3.27, P = 0.006]\) and markedly reduced the percentage of time spent in REM sleep \([t(23) = -8.02, P < 0.001]\). Furthermore, subjects in the amitriptyline group spent more time in stage 2 sleep \([t(23) = 4.11, P = 0.001]\). In contrast, subjects in the placebo group spent significantly more time awake \((U = 37, P = 0.03)\), which led to a lower total sleep time and thus also to a reduced sleep efficiency \([t(23) = 3.25, P = 0.006\) and \(t(23) = 3.03, P = 0.009\), respectively]. The increased time spent awake in the placebo group can be attributed to subjects needing significantly longer to fall back asleep after being awakened for medication administration \((U = 31, P = 0.009)\). Even if we ignore the length of time that subjects needed to fall back asleep after being awakened, the placebo group spent more time awake, had a greater number of awakenings during the night, and showed higher sleep latency, although these differences failed to reach statistical significance \((all P > 0.05)\). It would thus seem that the placebo group was negatively affected by the nocturnal awakening, whereas the amitriptyline group benefited from the sedative effect of amitriptyline, resulting in quantitative differences in sleep. The percentage of time spent in stage 1 sleep or in SWS did not differ between the two groups \((both P > 0.19; see Table 1 for details)\).

ANOVA showed a significant interaction effect between performance on the visual discrimination task and treatment \([F(1, 23) = 4.99, P = 0.04]\). Performance decreased under amitriptyline \((i.e., the perception threshold increased from training to retrieval)\) and improved under placebo \((Figure 2A)\). On the finger tapping task, the amitriptyline group showed larger gains in the number of correctly tapped sequences \((Figure 2B)\) and a greater reduction in error rates \((Figure 2C)\); group effects, however, failed to reach statistical significance \((both \(P > 0.19)\).
Ps > 0.60). On the two declarative memory tasks, the changes in performance in both groups were comparable (Figure 2D-E; see Table 2 for details). Performance during training did not differ significantly between the two groups on any of the memory tests (all Ps > 0.12).

**Discussion**

This is the first study to show that the tricyclic antidepressant amitriptyline impairs the consolidation of perceptual skill learning in healthy young subjects. This impairment cannot be attributed to a hangover effect during retrieval testing because declarative learning and motor skill learning were unaffected. Our findings support the hypothesis that REM-sleep-dependent procedural memory consolidation is impaired by suppressing REM sleep with an anticholinergic antidepressant, but not by doing so with an SSRI or SNRI. It would thus seem that procedural memory consolidation is not facilitated by the characteristics of REM sleep captured by visual sleep stage scoring, but rather by the high cholinergic tone associated with REM sleep. The notion that intact cholinergic transmission is a prerequisite for memory consolidation during REM sleep is consistent with recent findings: Blocking cholinergic transmission with scopolamine during the REM sleep window impaired procedural learning in rats (26), and blocking cholinergic transmission with scopolamine and mecamylamine during late nocturnal sleep, which is rich in REM sleep, impaired procedural memory consolidation in humans too (15). Similarly, cholinergic stimulation with an acetylcholine esterase inhibitor in another study improved REM-sleep-dependent procedural memory consolidation in healthy older adults (16).

Furthermore, different types of antidepressants appear to have different effects on motor skill learning, which has been associated with stage 2 sleep (18). Although another study showed improvements in motor skill memory after REM sleep was suppressed with the SSRI fluvoxamine or the SNRI reboxetine (11), our study did not show any significant differences between the amitriptyline and placebo groups. Considering that the receptor profile of
amitriptyline has several affinities (i.e., in addition to a strong affinity for cholinergic receptors, there are also affinities for serotonin and norepinephrine receptors), the lack of group differences in our study may reflect an interaction between the motor skill impairment resulting from the anticholinergic effect of amitriptyline and the motor skill enhancement resulting from this agent’s action on serotonin and norepinephrine receptors.

Importantly, amitriptyline did not affect sleep-dependent declarative memory consolidation in our study. This is in line with the finding that blocking muscarinic and nicotinic cholinergic receptors (or muscarinic cholinergic receptors alone) has no effect on declarative memory consolidation in healthy subjects (15, 27). Our finding that the anticholinergic antidepressant amitriptyline does not impair declarative memory consolidation is important considering that a recent study has suggested that depression may double the risk of developing Alzheimer’s disease (28) and that Alzheimer’s disease has been associated with cholinergic deficiency (29) and a decline in memory function, particularly in declarative memory.

Our study has several limitations. First, our subjects were awakened in order to receive their second dose of amitriptyline or placebo. Although neither SWS nor REM sleep awakenings have been shown to affect sleep-dependent memory consolidation (30), we cannot exclude the possibility that waking subjects during stage 2 sleep may have had an effect on our findings. Second, whereas the amitriptyline group benefited from the sedative effect of amitriptyline, the placebo group was negatively affected by the nocturnal awakening, leading to a higher amount of time awake, lower total sleep time, and reduced sleep efficiency likely due to the laboratory condition. As a result, the improvement in the memory tasks seen in the placebo group during retrieval testing might have been higher in the absence of a nocturnal awakening. Third, amitriptyline was administered for only one night. Sleep architecture, and thus memory consolidation, may differ with long-term antidepressant use, depending on the agent and its dosage. Finally, because our subjects were healthy young men, results cannot easily be generalized to depressed patients or even healthy females, particularly to those
belonging to different age groups. Whereas one study has shown that motor skill memory in healthy subjects was not affected by an SSRI or an SNRI (11), it has been found to be impaired in depressive patients on various antidepressive agents (8, 9). Moreover, sleep-dependent skill memory consolidation has been found to be preserved in young patients but was severely impaired in older ones (9, 31). Further research on the possible side effects of antidepressants should thus take different age groups into account.

In conclusion, our results show that the antidepressant amitriptyline impaired the sleep-dependent consolidation of a perceptual skill in healthy young subjects, while the consolidation of a motor skill and declarative memory consolidation remained unaffected. This is the first study to show that an anticholinergic antidepressant can have this negative effect at least in healthy men. Because antidepressants are the most commonly prescribed class of medication in the United States and depression is associated with a two-fold risk of developing dementia, prospective studies are needed to determine whether antidepressants have a negative impact on various memory systems and, if so, under which circumstances.

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References


Table Legends

Table 1. Sleep parameters after administration of 75 mg amitriptyline or placebo

“Time in bed” (TIB) was defined as time from lights off to lights on; “sleep period time” (SPT) as the first epoch of stage 2 sleep to the first epoch of wake without a subsequent epoch of sleep; “total sleep time” (TST) as SPT minus wake after sleep onset; “sleep latency” as time from lights off to the first epoch of stage 2 sleep; “REM latency” as time from the first epoch of stage 2 sleep to the first epoch of REM sleep; “sleep efficiency” as the ratio of TST to TIB; “awakenings” as the sum of periods with at least one epoch awake during SPT; “latency after awakening” as minutes needed to reach sleep stage 2, 3, 4, or REM again after being awakened for medication administration; “wake without latency after awakening” as minutes spent awake during SPT minus latency after awakening. Periods spent awake, in stage 1, 2, slow-wave sleep (SWS), or rapid eye movement (REM) sleep are given in minutes or as percentage of SPT. Means and SEMs are shown. The rightmost column indicates $P$ values for pairwise comparisons between the amitriptyline and placebo groups (unpaired $t$-test or exact Mann-Whitney $U$ tests).

Table 2. Memory performance during training and retrieval testing 24 hours later

Means and SEMs are shown. The rightmost column indicates $P$ values for pairwise comparisons between the amitriptyline and placebo groups (unpaired $t$-test or exact Mann-Whitney $U$ tests).
Figure Legends

Figure 1. Experimental procedure
In a double-blind, parallel-group design, we investigated the effects of amitriptyline \( n = 12 \) versus placebo \( n = 13 \) on sleep-dependent memory consolidation. Memory was tested using visual discrimination task, finger tapping, the Rey Auditory-Verbal Learning Test, and the Rey-Osterrieth Complex Figure Test. Medication was administered on two separate occasions after training. Retrieval testing took place 24 hours after training.

Figure 2. Main results
(A) The amitriptyline and placebo groups differed significantly between training and retrieval in their performance on the visual discrimination task. Performance decreased in the amitriptyline group but improved in the placebo group. In contrast, no significant differences were observed in the performance of the two groups on (B-C) the finger tapping task, (D) the Rey-Osterrieth Complex Figure Test, or (E) the Rey-Auditory Verbal Learning Test. Means and SEMs are shown.

Figure 3. Stimulus and mask patterns in the visual discrimination task
(A) Stimulus pattern: display was 14° of visual angle in size, containing a field of 19 x 19 horizontal bars with a rotated “T” or “L” at its center. The target texture, which consisted of three horizontally or vertically aligned diagonal bars, was varied randomly from trial to trial but always in the same quadrant and at a distance of 3° to 5° of visual angle from the center of the display. (B) Mask pattern: consisted of randomly oriented “V” and a central “T-L” mix.