Combined Blockade of Cholinergic Receptors
Shifts the Brain from Stimulus Encoding
to Memory Consolidation

Björn H. Rasch, Jan Born, and Steffen Gais

Abstract
High central nervous system levels of acetylcholine (ACh) are commonly regarded as crucial for learning and memory, and a decline in cholinergic neurotransmission is associated with Alzheimer’s dementia. However, recent findings revealed exceptions to this rule: The low ACh tone characterizing slow-wave sleep (SWS) has proven necessary for consolidation of hippocampus-dependent declarative memories during this sleep stage. Such observations, together with recent models of a hippocampal–neocortical dialogue underlying systems memory consolidation, suggest that high levels of ACh support memory encoding, whereas low levels facilitate consolidation. We tested this hypothesis in human subjects by blocking cholinergic neurotransmission during wakefulness, starting 30 min after learning. Subjects received the muscarinic antagonist scopolamine (4 μg/kg bodyweight intravenously) and the nicotinic antagonist mecamylamine (5 mg orally). Compared to placebo, combined muscarinic and nicotinic receptor blockade significantly improved consolidation of declarative memories tested 10 hr later, but simultaneously impaired acquisition of similar material. Consolidation of procedural memories, which are not dependent on hippocampal functioning, was unaffected. Neither scopolamine nor mecamylamine alone enhanced declarative memory consolidation. Our findings support the notion that ACh acts as a switch between modes of acquisition and consolidation. We propose that the natural shift in central nervous system cholinergic tone from high levels during wakefulness to minimal levels during SWS optimizes declarative memory consolidation during a period with no need for new memory encoding.

INTRODUCTION
The cholinergic system modulates brain activity through a network of neural fibers originating in the basal forebrain and tegmental regions and spreading to the entire neocortex, amygdala, and hippocampus (Everitt & Robbins, 1997). General central nervous system cholinergic activity reaches a minimum during slow-wave sleep (SWS), whereas wakefulness is marked by high levels of acetylcholine (ACh) activity (Pace-Schott & Hobson, 2002). High levels of brain ACh activity are considered a prerequisite for efficient memory function during the wake phase (Bartus, 2000; Bartus, Dean, Beer, & Lippa, 1982). Accordingly, memory deficits in patients with Alzheimer’s disease, who show reduced ACh activity, are commonly treated with ACh-enhancing drugs (Scarpini, Scheltens, & Feldman, 2003).

The formation of long-term memories requires a process of consolidation, which is facilitated by sleep (Walker & Stickgold, 2004; Maquet, 2001; McGaugh, 2000). In the case of hippocampus-dependent declarative memories, the consolidation process benefits particularly from SWS (Gais & Born, 2004a; Marshall, Molle, Hallschmid, & Born, 2004; Molle, Marshall, Gais, & Born, 2004; Peigneux et al., 2004; Lee & Wilson, 2002; Plihal & Born, 1997). Recent experiments in humans indicate that raising the cholinergic tone during a period of SWS-rich sleep by administering the cholinesterase inhibitor physostigmine completely eliminated this sleep-related declarative memory formation (Gais & Born, 2004b). This observation fits well with recent concepts of a hippocampal–neocortical dialogue proposed by Buzsaki (1996, 1989) and Hasselmo (1999), according to which low ACh activity sets the appropriate dynamics for memory consolidation by disinhibition of intrahippocampal and hippocampal–neocortical feedback synapses. This disinhibition allows spontaneous reactivation of hippocampal memory traces and their transfer to neocortical networks. In contrast, high cholinergic tone during wakefulness ensures effective acquisition by suppressing feedback activity and hippocampal memory reactivation that could otherwise interfere with ongoing encoding. Although this concept has received strong support mainly from in vitro studies (Barkai & Hasselmo, 1994; Pitler & Alger, 1992), behavioral evidence for the suggested role of ACh levels on consolidation and memory reactivation is rare (as reviewed in Atri et al., 2004;
It has to be noted that the above concept relates to systems memory consolidation (Dudai, 2004), specifically the transfer of hippocampal memory traces to the neocortex. Accordingly, from this perspective, early post-training processes, which stabilize synaptic changes directly after their induction, rather belong to ongoing encoding and will not be considered here.

In the present experiments, we tested the prediction that lowering cholinergic neurotransmission after learning during waking supports systems memory consolidation for hippocampus-dependent memory. To generally block cholinergic transmission during wakefulness, we administered a combination of the muscarinic receptor antagonist scopolamine and the nicotinic receptor antagonist mecamylamine to healthy young men 30 min after learning. The 30-min period immediately after learning was spared because during this time, processes more closely related to encoding may persist, and our intervention should target the level of systems consolidation. In a control condition, subjects received placebo. Participants learned a declarative word pair association task and a nondeclarative (i.e., procedural) finger sequence tapping task from 9 to 10 a.m. in the morning (learning). Recall was tested at 8 p.m., 10 hr after the end of the learning period (retrieval). In a supplementary experiment, we examined the effects of separate muscarinic or nicotinic cholinergic blockade on memory consolidation.

METHODS

Subjects

Eighteen men (mean age 24.7 years, range 21–29 years) participated in the main experiments. Subjects were healthy, nonsmoking, native-German-speaking, right-handed students. They underwent a routine physical and mental health examination, did not take any medication at the time of the experiments, and reported a normal sleep-wake cycle. One subject of the main experiments was replaced because he developed nausea after substance administration. The experiments were approved by the ethics committee of the University of Lübeck. Written informed consent was obtained from all subjects prior to participating.

Procedure

Each subject participated in two experimental sessions separated by an interval of at least 2 weeks according to a double-blind cross-over design. One session served to assess effects of cholinergic blockade and the other as placebo control condition. The order of conditions was balanced across subjects. Sessions started at 8:30 a.m. with a short interview to ensure that the subjects had slept normally on the nights before and had their usual breakfast, without coffee or black tea. Then, subjects had a venous catheter inserted for substance administration and blood collection. During the learning phase (9:00–10:00 a.m.), the participants completed two memory tasks, first a declarative word pair association task and then a procedural finger sequence tapping task. Thirty minutes after learning (10:30 a.m.), the substances were administered. In order to assess substance effects on acquisition, the subjects performed a number list learning task 1 hr after substance administration, at 11:30 a.m. (encoding test). During the entire retention interval, subjects remained in the presence of the experimenter and were allowed to play computer games and watch movies. Retrieval on the two memory tasks was tested 9.5 hr after substance administration, starting at 8:00 p.m. with the word pair task followed by the finger sequence tapping task. To control for effects of the pharmacological manipulation on retrieval function, subjects were then asked to recall another list of word pairs that they had learned 4 days prior to substance administration (test of retrieval function). Standardized light meals were provided at 12:30 p.m. and at 6:00 p.m. Every 2 hr, reaction time, mood, feelings of tiredness and calmness/restlessness, subjective symptoms, and blood pressure were monitored, and blood was sampled for determination of cortisol concentrations. A summary of the procedure is given in Figure 1.

Substance Administration

To block cholinergic transmission, we administered a combination of the muscarinic receptor antagonist scopolamine (4 µg/kg body weight intravenously over 20 min) and the nicotinic receptor antagonist mecamylamine (5 mg orally). Both substances were administered simultaneously at 10:30 a.m., 30 min after the end of the learning phase (Figure 1). We chose relatively low doses to avoid strong side effects of cholinergic blockade and to ensure that the substances had largely washed out at the time of retrieval testing. The half-life in plasma has been estimated at 4.5 ± 1.7 hr for scopolamine (Putcha, Cintron, Tsui, Vanderploeg, & Kramer, 1989) and 10.1 ± 2 hr for mecamylamine (Young, Shytle, Sanberg, & George, 2001). In the control condition, subjects received placebo.

Tasks

The word pair association task consisted of a list of 40 pairs of semantically related words (e.g., clock–church). Four different lists were constructed and balanced according to ratings of concreteness, meaningfulness, imagery, valence, arousal, and association strength. One list was used in each session. The two remaining lists were used for testing long-term memory-retrieval function...
and were learned 4 days before the respective experimental sessions. During the learning phase, the word pairs were presented sequentially on a computer screen, each for 5 sec, separated by interstimulus intervals of 100 msec. After the entire list was finished, performance was tested by using a cued recall procedure; that is, the first word (cue) of each pair was presented and the subject had to name the associated second word (response). The correct response word was then displayed for 2 sec, regardless of whether the response was correct or not, to allow re-encoding of the correct word pair. This cued recall procedure was repeated until the subject reached a criterion of 60% correct responses. The learning criterion for the lists learned 4 days before the main session was 70%. Retrieval was tested at the end of the experimental session with the same cued recall procedure as during the learning phase. Lists were always presented in randomized order. As dependent variable, we used the number of correctly recalled words at retrieval relative to the number of correctly recalled words during the learning period. Note that this measure can result in values higher than 100% if subjects recall more words during the retrieval as compared to the learning period.

In the number list learning task, 16 two-digit numbers ranging from 12 to 99 were presented sequentially for 2 sec in randomized order, with an interstimulus interval of 500 msec. Two lists of numbers that contained the same amount of numbers with repeating digits (e.g., 33, 66) were used. The complete list was presented four times. After 1 min, recognition was tested by presenting all 16 numbers of the old list randomly interspersed among 16 new numbers. The subject had to decide whether a number belonged to the old list.

Measurement of Further Physiological and Psychological Symptoms of Cholinergic Blockade

Reaction times were assessed by a standardized test that required pressing a button as fast as possible whenever a big red disc appeared on a computer screen (Little, Johnson, Minichiello, Weingartner, & Sunderland, 1998). On 40 trials, the subject fixed their gaze on a cross displayed for 500–1000 msec on a white screen. Then, in 35 trials, a red disc appeared, and in 5 random no-go trials, the screen remained white.

Mood and feelings of tiredness and of calmness/restlessness were assessed by using the short form of the German version of the Multidimensional Mood Questionnaire (Steyer, Schwenkmezger, Notz, & Eid, 1994). The subjects indicated on a 5-point rating scale how well 12 different adjectives described their current feeling. The adjectives were assigned to one of three different bipolar dimensions, pleasant/unpleasant, alert/tired, and calm/restless, with values between 4 and 20. In addition, subjects were asked to report any unusual symptoms they experienced.

Blood pressure was measured with a digital blood pressure meter (Boso-Medicus, Bosch & Sohn GmbH, Jungingen, Germany). For determination of cortisol concentrations, blood samples were immediately centrifuged, and serum was stored at −20°C until standard assay (Immulite, DPC Biemann, Bad Nauheim, Germany). To obtain estimates of the nonspecific symptoms after cholinergic blockade during the learning and the
retrieval periods, the values directly before and after the respective periods were averaged (Figure 1).

**Supplementary Experiments**

Fifteen men (mean age 28.2 years; range 23–34 years) were examined in a supplementary experiment. Subjects were tested on three occasions. In one condition, they received a placebo (saline solution, 10 ml intravenously, and a vehicle tablet); in another, the muscarinic antagonist scopolamine (4 μg/kg bodyweight in 10 ml saline solution intravenously over 20 min plus a vehicle tablet); and in a third condition, the nicotinic antagonist mecamylamine (5 mg orally plus saline solution, 10 ml intravenously over 20 min). Substances were administered according to a balanced double-blind cross-over design. Procedures of the supplementary experiments were identical to that of the main experiment, except that the number list learning task and long-term retrieval testing of word pairs learned 4 days before were omitted. In the word pair association task, the four word lists used in the main experiment were assigned in a balanced way to the three experimental sessions. In the finger sequence tapping task, two additional sequences were used (4-3-1-2-4 and 4-2-1-3-4), so that a total of four sequences were assigned in a balanced way to the subjects’ three sessions.

**RESULTS**

The main finding of this study is that declarative memory for the word pairs was improved 10 hr after acquisition when cholinergic neurotransmission was blocked during the consolidation period. With combined scopolamine/mecamylamine administration after learning, subjects recalled 98.9 ± 2.8% of the word pairs they had successfully learned before substance administration, whereas with placebo they remembered only 89.9 ± 2.9%, \( F(1,17) = 7.68, p = .01 \) (Figure 2A). Learning
before substance administration did not differ between conditions. Absolute numbers of correctly recalled words during learning and retrieval periods are shown in Table 1.

Increasing the low level of ACh activity by administration of physostigmine during a 3-hr retention period of predominant SWS in a previous study diminished memory for word pairs by 12.2 ± 2.8% (Figure 2B, Gais & Born, 2004b). To assess whether the influence of cholinergic tone was comparable in sleeping and awake subjects, we performed a combined analysis on those and the present data. Results confirmed a highly significant main effect of low (SWS and ACh receptor blockade in waking subjects) versus high cholinergic tone (physostigmine during SWS and placebo in awake subjects), $F(1,27) = 20.27, p = .001$. Notably, this effect did not depend on sleep or wakefulness, $F(1,27) = 0.44, p > .50$, for Cholinergic Tone × Sleep/Wake interaction.

To show that ACh receptor blockade has differential effects on encoding compared to consolidation of declarative memories, participants performed a number learning task 1 hr after substance administration (encoding test, see Figure 1). After combined scopolamine/mecamylamine administration, subjects recognized distinctly fewer numbers than after placebo ($p = .02$, Figure 2F). In view of the presence of clear symptoms of cholinergic blockade, such as dizziness, tiredness, restlessness, dryness of the mouth, increased reaction time, and reduced blood pressure, at the time of the number list learning task (Figure 3), we examined a possible contribution of these symptoms to the impairment in encoding but found no significant correlation.

Virtually all the symptoms of cholinergic blockade had disappeared at the time of retrieval testing 9.5 hr after substance administration (Figure 3). There was no difference between placebo and scopolamine/mecamylamine conditions at this time with respect to reaction times, subjective mood, alertness, or cortisol concentration ($p > .10$, for all comparisons). Only systolic and diastolic blood pressure were still decreased after combined cholinergic blockade at the end of the session ($p < .05$, Figure 3E and F). However, this effect on blood pressure at retrieval was not significantly correlated with the observed effect on word pair recall after cholinergic receptor blockade. In fact, respective coefficients were even negative; that is, large differences in blood pressure were associated with small differences in word pair recall and vice versa ($R = -.18$ and $R = -.30$, for systolic and diastolic blood pressure, respectively; $p > .20$). In order to further exclude that the symptoms of cholinergic blockade affected word pair retrieval rather than memory consolidation, subjects had to retrieve a second list of word pairs, which they had learned 4 days earlier. Recall of this list was comparable for the scopolamine/mecamylamine and placebo conditions ($p > .30$; Figure 2E and Table 1).

The enhancing effect of combined ACh receptor blockade in the awake subjects was specific to the consolidation of hippocampus-dependent declarative memory. Speed and accuracy in the procedural finger sequence tapping task were not affected by this blockade after the retention interval ($p > .20$; Figure 2D and Table 1).

Supplementary experiments revealed that unlike combined ACh receptor blockade, selective blocking of either muscarinic or nicotinic receptors alone after learning did

### Table 1. Performance at Learning and at Retrieval on Word Pair and Finger Sequence Tapping Tasks

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Scopolamine + Mecamylamine</th>
<th>$F(1,17)$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Word pairs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of trials to criterion (60%)</td>
<td>Learning 1.6 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>0.00</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Number of recalled word pairs</td>
<td>Learning 29.8 ± 0.9</td>
<td>30.4 ± 0.8</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Retrieval 26.8 ± 1.1</td>
<td>29.9 ± 0.8</td>
<td>8.42</td>
<td>≤.01</td>
</tr>
<tr>
<td><strong>Finger sequence tapping</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed (total number of sequences)</td>
<td>Learning 15.8 ± 1.0</td>
<td>16.4 ± 1.1</td>
<td>2.77</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Retrieval 16.1 ± 1.1</td>
<td>16.1 ± 1.2</td>
<td>0.00</td>
<td>ns</td>
</tr>
<tr>
<td>Error rate (% sequences with errors)</td>
<td>Learning 9.7 ± 2.2</td>
<td>9.4 ± 2.3</td>
<td>0.01</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Retrieval 9.5 ± 2.1</td>
<td>6.5 ± 1.2</td>
<td>2.76</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Retrieval function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of recalled word pairs</td>
<td>Retrieval 16.0 ± 1.4</td>
<td>17.6 ± 1.6</td>
<td>0.75</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are means ± SEM. For the learning phase, the number of trials needed to reach the learning criterion is indicated. Retrieval function was tested by recall of word pairs learned 4 days before. Right columns: $F$ and $p$ values for comparison between treatments.
not improve consolidation of declarative memory ($p > .40$; Figure 2C). Procedural memory consolidation was unaffected by the treatment as well ($p > .10$; Table 2).

**DISCUSSION**

The novel finding of our experiments in humans is that reduced global cholinergic neurotransmission during wakefulness selectively improves consolidation of hippocampus-dependent declarative memories, whereas encoding is impaired at the same time. As previously reported, increasing the low level of ACh activity during a period of predominant SWS by physostigmine administration diminished memory for word pairs by a difference similar to that seen here in the awake subjects between the conditions of low and high ACh activity (Gais & Born, 2004b). Taken together, the data indicate that a low level of ACh neurotransmission is crucial for declarative memory consolidation, which is naturally occurring during sleep. The results support recent concepts of hippocampal memory, which propose that systems consolidation of memories encoded during wakefulness requires a shift toward lower cholinergic tone (Dudai, 2004; Hasselmo & McGaughy, 2004; Buzsaki, 1996). Specifically, it is assumed that lowered cholinergic activity enables spontaneous replay of activity in the hippocampal CA3 neuronal assemblies by disinhibiting feedback connections within this area. Moreover, reduced cholinergic tone releases inhibition from connections to the CA1, entorhinal cortex, and neocortex, thereby enabling
transfer of information from hippocampal regions to neocortical networks (Hasselmo, 1999). In this way, neocortical memory traces are thought to be strengthened by hippocampal replay activity and to become linked to other memories, resulting in an integration and consolidation of information (Wagner, Gais, Haider, Verleger, & Born, 2004; McClelland, McNaughton, & O'Reilly, 1995). However, a global blockade of cholinergic neurotransmission, as achieved in our experiments, may similarly change the activity of other neuronal circuitry, including the amygdala and neocortex. These changes may have contributed as well to the observed memory effects.

Our experiments not only indicate that reduced cholinergic neurotransmission can facilitate consolidation processes during waking, but they also show that a substantial decrease in ACh tone during waking would prevent efficient encoding under normal conditions. Thus, sleep represents a period where the various processes that optimize declarative memory consolidation, including a drop in ACh levels, are established without interfering with cognitive processing demands characterizing the wake phase.

Our finding that cholinergic blockade impairs memory encoding is in line with those of many previous studies (see, e.g., Rogers & Kesner, 2003; Sherman, Atri, Hasselmo, Stern, & Howard, 2003; Hasselmo & Wyble, 1997). Some have argued that encoding impairments after cholinergic blockade are partly due to nonspecific symptoms following substance administration (Blokland, 1995); however, our analysis does not show any correlation of encoding deficits with other nonspecific effects. A contribution of nonspecific effects is further ruled out in light of studies testing cholinergic antagonists that act only peripherally and had no effect on memory encoding (see Collerton, 1986, for a review). On the other hand, encoding is clearly impaired when cholinergic antagonists are administered directly into the hippocampus (Marti, Ramirez, Dos Reis, & Izquierdo, 2004; Wallenstein & Vago, 2001). The improvement in declarative memory performance after combined cholinergic receptor blockade cannot be reduced to an influence of the cholinergic antagonists on retrieval function. At the time of retrieval testing 10 hr after learning, virtually all other effects and nonspecific symptoms of cholinergic blockade had disappeared. Moreover, word pairs that had been learned 4 days before the experiments (so that respective memory traces were already consolidated to a great extent at the time of the experiments) were recalled equally well after placebo as after combined cholinergic blockade. This confirmed that retrieval function was closely comparable between both treatment conditions.

The finding that selective postlearning blockade of either muscarinic or nicotinic ACh receptors alone does not influence the consolidation of declarative memories and their later recall confirms a number of previous human studies (Newhouse, Potter, Corwin, & Lenox, 1994; Mewaldt & Ghoneim, 1979; Petersen, 1977; Ghoneim & Mewaldt, 1975; Safer & Allen, 1971). In animal studies, blocking muscarinic or nicotinic receptors separately after learning had either no effect (Miranda, Ferreira, Ramirez-Lugo, & Bermudez-Rattoni, 2003; Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999; Rush, 1988; Spangler, Chachich, & Ingram, 1988) or impaired memory consolidation, although only at doses approximately 10 times higher than those sufficient to cause encoding deficits (Marti et al., 2004; Schildein, Huston, & Schwarting, 2002; Wallenstein & Vago, 2001; Schildein, Huston, & Schwarting, 2000; Roldan, Bolanos-Badillo, Gonzalez-Sanchez, Quirarte, & Prado-Alcala, 1997; Rudy, 1996; Flood & Cherkin, 1986). Combined muscarinic and nicotinic blockade was less frequently examined. With regard to

Table 2. Performance at Learning and at Retrieval on Word Pair and Finger Sequence Tapping Tasks in the Supplementary Experiment (Separate Administration of Scopolamine and Mecamylamine)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Mecamylamine</th>
<th>Scopolamine</th>
<th>F(2,28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Word pairs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of trials to criterion (60%)</td>
<td>Learning</td>
<td>2.6 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Learning</td>
<td>28.1 ± 0.9</td>
<td>28.2 ± 0.9</td>
<td>28.5 ± 0.9</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Retrieval</td>
<td>25.4 ± 1.4</td>
<td>24.7 ± 1.0</td>
<td>26.2 ± 1.3</td>
<td>1.10</td>
</tr>
<tr>
<td><strong>Finger sequence tapping</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed (total number of sequences)</td>
<td>Learning</td>
<td>14.2 ± 1.3</td>
<td>14.4 ± 0.8</td>
<td>14.4 ± 1.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Retrieval</td>
<td>14.0 ± 1.1</td>
<td>13.6 ± 0.9</td>
<td>14.1 ± 1.4</td>
<td>0.25</td>
</tr>
<tr>
<td>Error rate (% sequences with errors)</td>
<td>Learning</td>
<td>15.5 ± 3.7</td>
<td>13.9 ± 2.6</td>
<td>9.5 ± 1.9</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>Retrieval</td>
<td>11.0 ± 2.3</td>
<td>11.4 ± 1.6</td>
<td>12.5 ± 3.0</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Data are means ± SEM. For the learning phase, the number of trials needed to reach the learning criterion is indicated. Right columns: F and p values for comparison between treatments.
encoding, the effects of scopolamine and mecamylamine were additive in these experiments (Levin, McGurk, South, & Butcher, 1989). However, with regard to memory consolidation, the impairing effects observed for each of the substance with postlearning administration appeared to neutralize each other when the substances were administered in combination (Maviel & Durkin, 2003; Glick & Greenstein, 1972). Notably, employing a Morris water maze task sharing the hippocampal dependence of the declarative tasks used here, Cozzolino et al. (1994) found that conjoint blocking of muscarinic and nicotinic receptors in fact enhanced long-term retention on this task in a dose-dependent manner.

A straightforward comparison of the effects of combined cholinergic receptor blockade with those of selective blockade of muscarinic receptors is hampered by the fact that scopolamine, by blocking M2 presynaptic autoreceptors, induces a pronounced increase in ACh release, which, in turn, can lead to an overactivation of postsynaptic nicotinic receptors (Maviel & Durkin, 2003; Durkin, Messier, de Boer, & Westerink, 1992). Hence, modeling the effects of a generally lowered cholinergic neurotransmission, as naturally observed during SWS, definitely requires simultaneously blocking of both cholinergic receptor types, thereby avoiding increased nicotinic activation. However, it remains to be added that due to the low dose of scopolamine used here and its only moderate affinity to the M2 autoreceptor, the increase in extracellular ACh after scopolamine was presumably small (Byster, Heath, Hendrix, & Shannon, 1993). It should be noted that due to the different routes of administration and half-lives of the substances used in our experiment, blockade of nicotinic receptors presumably started later and lasted longer than blockade of muscarinic receptors. Because neither scopolamine nor mecamylamine alone affected declarative memory consolidation, this transient imbalance in receptor blockade per se probably cannot explain our results. Still we cannot entirely exclude that this special time course of receptor blocking contributed to the influence on memory consolidation.

Whereas in most other studies cholinergic activity was blocked immediately after learning, in our study we administered the ACh receptor antagonists not until 30 min after the end of the learning phase. Ragozzino, Pal, Unick, Stefani, and Gold (1998) showed that learning of a spontaneous alteration task induced an increase in hippocampal ACh levels that persisted for up to 20 min after training and may serve to further enhance synaptic processes (Chang & Gold, 2003; Power, Vazdarjanova, & McGaugh, 2003; Rasmusson, 2000). In addition, long-term potentiation, an experimental phenomenon that is considered to be analogous to memory, becomes stable only after about 30 min (Lynch, 2004; Izquierdo & McGaugh, 2000). Blocking cholinergic neurotransmission immediately after learning might therefore interfere with neuronal processes needed for completing successful encoding. Because we spared this early period after encoding, our data must be interpreted as an improving effect on systems consolidation of declarative memories that requires a global suppression of cholinergic neurotransmission.

In conclusion, as predicted from recent models of hippocampal–neocortical memory processing, combined nicotinic and muscarinic blockade after learning improved declarative memory consolidation compared to placebo and simultaneously impaired memory encoding of similar material. Our results imply that SWS is a natural condition of optimized memory consolidation because cholinergic tone is minimal during this sleep stage. It can also be assumed that ACh-enhancing drugs, often used to treat Alzheimer’s disease, will benefit encoding, but will impair consolidation of declarative memories.

Acknowledgments
We thank A. Otterbein for technical assistance. This work was supported by grants from the Deutsche Forschungsgemeinschaft (to S. G.) and from the Volkswagen foundation (to J. B.). The authors declare that they have no competing financial interest.

Reprint requests should be sent to Jan Born, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany, or via e-mail: born@kfg.uni-luebeck.de.

REFERENCES


