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Blockade of Central Cholinergic Receptors Impairs New Learning and Increases Proactive Interference in a Word Paired-Associate Memory Task

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Experimental data and computational models suggest that blockade of muscarinic cholinergic receptors impairs paired-associate learning and increases proactive interference (E. DeRosa & M. E. Hasselmo, 2000; M. E. Hasselmo & J. M. Bower, 1993). The results presented here provide evidence in humans supporting these hypotheses. Young healthy subjects first learned baseline word pairs (A–B) and, after a delay, learned additional overlapping (A–C) and nonoverlapping (D–E) word pairs. As predicted, when compared with subjects who received the active placebo glycopyrrolate (4 μg/kg) and subjects who were not injected, those who received scopolamine (8 μg/kg) showed (a) overall impairment in new word paired-associate learning, but no impairment in cued recall of previously learned associates; and (b) greater impairment in learning overlapping (A–C) compared with nonoverlapping (D–E) paired associates.

Acetylcholine may play an important role in the encoding of new information. Numerous studies demonstrate that blockade of muscarinic acetylcholine receptors by systemic administration of the drug scopolamine interferes with the encoding of new verbal information, but has little effect on retrieval of previously stored information (Beatty, Butters, & Janowsky, 1986; Crow & Grove-White, 1973; Drachman, 1978; Frith, Richardson, Samuel, Crow, & McKenna, 1984; Ghoneim & Mewaldt, 1975, 1977; Hasselmo, 1995; Hasselmo & Wyble, 1997; Mewaldt & Ghoneim, 1979; Peterson, 1977; Troster, Beatty, Staton, & Rorabaugh, 1989). Until

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recently, most human studies with scopolamine had primarily demonstrated its effects on recollection memory (i.e., recall of newly acquired information after at least a short delay) and its sparing of semantic and procedural memory (Broks et al., 1988; Caine, Weingartner, Ludlow, Cudahy, & Wehry, 1981; Nissen, Knopman, & Schacter, 1987). In addition, short-term memory phenomena such as the recency component of a serial position curve (Crow & Grove-White, 1973; Frith et al., 1984) and digit span (Beatty et al., 1986; Drachman, 1978) are also spared. Recently, studies have shown that scopolamine may impact recognition memory processes by affecting both recollection and familiarity (Mintzer & Griffiths, 2001, 2003; Sherman, Atri, Hasselmo, Stern, & Howard, 2003). These studies suggest that the effects of scopolamine may particularly affect the hippocampus and parahippocampal structures, as lesions of these structures cause a similar pattern of deficits (impaired recognition and recall of previously experienced events, with sparing of semantic memory, procedural memory, and short-term memory; Baddeley & Warrington, 1970; Corkin, 1984; Graf, Squire, & Mandler, 1984). To our knowledge, there have been no studies that directly examined the mnemonic effects of scopolamine on learning of predefined novel word-pair associates.

A selective impairment of encoding induced by scopolamine has also been demonstrated in experiments testing episodic memory for visual objects in monkeys (Aigner, Walker, & Mishkin, 1991) and episodic memory for platform location in the Morris water maze in rats (Sutherland, Whishaw, & Regehr, 1982; Whishaw, 1985). Taken together, these data in humans and animals suggest that acetylcholine normally sets neural dynamics that enhance the encoding of new information (Hasselmo, 1999).

Computational modeling has also been used to analyze the behavioral effects of cholinergic blockade in terms of the normal cholinergic modulation of cellular physiology (Hasselmo, 1999; Hasselmo & Bower, 1993; Hasselmo & Linster, 1998; Hasselmo & Wyble, 1997; Hasselmo, Wyble, & Wallenstein, 1996). The impairment of encoding described previously could result from blockade of the cholinergic enhancement of long-term potentiation (Blitzer, Gil, & Landau, 1990; Burgard & Sarvey, 1990; Hasselmo & Barkai, 1995; Huerta & Lisman, 1993; Patil, Linster, & Lubenov, & Hasselmo, 1998), which would result in weaker synaptic connections mediating retrieval (Hasselmo & Wyble, 1997). In addition, computational modeling suggests other effects of cholinergic blockade. In particular, acetylcholine normally suppresses excitatory synaptic transmission at intrinsic and feedback connections within cortical structures (Hasselmo & Bower, 1992; Hasselmo & Schnell, 1994; Hasselmo, Schnell, & Barkai, 1995; Herreras, Solis, Munoz, Martin del Rio, & Lerma, 1988; Hounsgaard, 1978; Linster, Wyble, & Hasselmo, 1999; Valentino & Dingledine, 1981). When acetylcholine levels are lower, or when muscarinic receptors are blocked, synaptic transmission at these connections becomes stronger (Hasselmo, 1999; Herreras, Solis, Herranz, Martin del Rio, & Lerma, 1988; Winson & Abzug, 1977). As illustrated schematically in Figures 1 and 2, computational modeling demonstrates that the cholinergic suppression of excitatory transmission could prevent retrieval of previously encoded information from interfering with the encoding of new information (Hasselmo & Bower, 1993; Hasselmo et al., 1996). Blockade of cholinergic suppression should enhance the excitatory feedback, and should thereby increase proactive interference caused by previously encoded information (Hasselmo et al., 1996; Hasselmo & Wyble, 1997). In previous studies of olfactory processing in rats, blockade of cholinergic receptors by scopolamine has been shown to impair learning of the responses to overlapping, but not nonoverlapping, odor pairs (DeRosa & Hasselmo, 2000).

In the study presented here, we used methods and data analysis techniques similar to those of DeRosa and Hasselmo (2000) to test predictions of the model by examining scopolamine's effects on memory for word-pair associates in humans. DeRosa and Hasselmo used adult rats performing a simultaneous odor discrimination task to show that rats receiving scopolamine were more

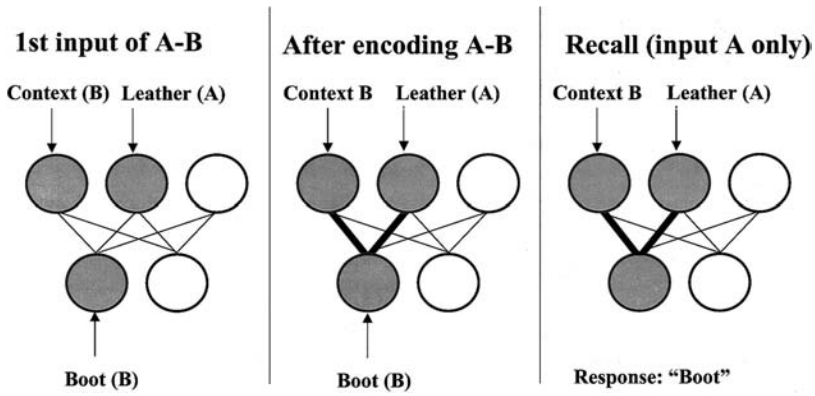

Figure 1. Computational modeling of the encoding and retrieval of paired associates in cortical structures. Each circle represents a population of cortical neurons, with gray shading representing neuronal activity, and the width of lines representing strength of excitatory feedback synapses between these populations. Left: During encoding, external input activates a population of neurons representing the words *leather* and *boot*, along with neurons representing the experimental context. Middle: Hebbian synaptic modification results in strengthening of connections between active neurons, as shown by thickening of lines. Right: During recall, external input activates the neurons representing the word *leather* and the experimental context. Activity spreads across previously modified synapses to activate the neurons representing the word *boot.*

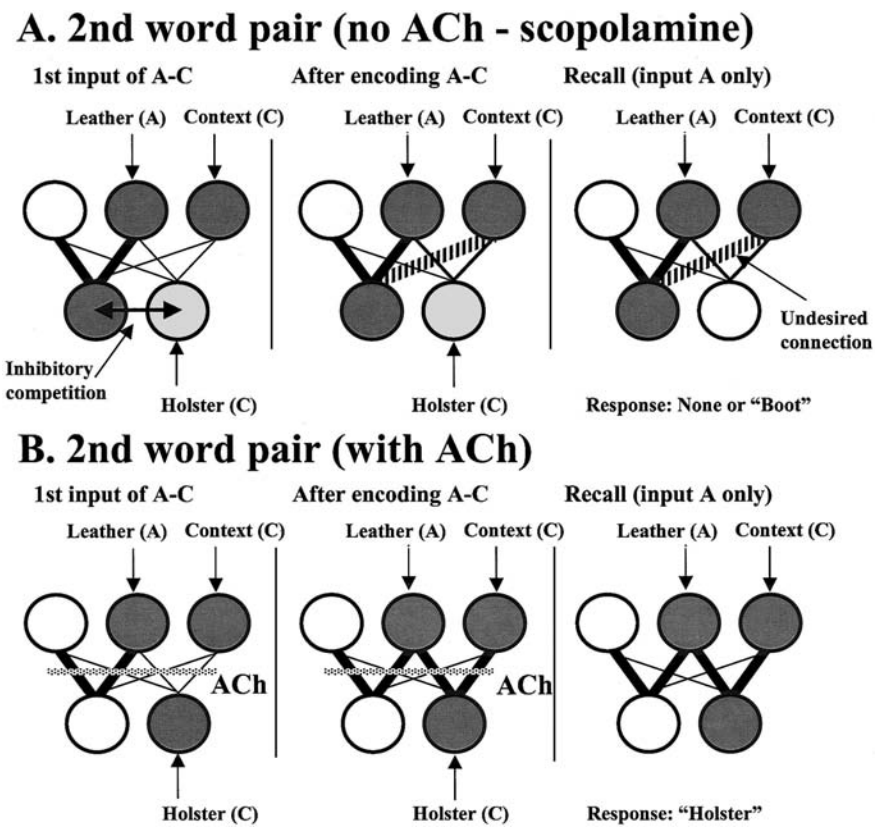

Figure 2. Demonstration of how cholinergic blockade of muscarinic suppression can enhance proactive interference. Both examples show encoding of a second overlapping word pair after encoding of the first pair shown in Figure 1. A: Example of network function with scopolamine blocking the cholinergic suppression of synaptic transmission. Left: During learning of the second word pair (*leather–holster*), activity spreads across the previously modified synapse to activate the neurons representing the word *boot.* Inhibitory competition reduces activity of the population of neurons representing the word *holster.* Middle: Reduced activity of neurons representing *holster* results in less strengthening of input to these neurons. Also, Hebbian synaptic modification strengthens the undesired connection between the second context (Context C) and the first word (dashed line). Right: During retrieval, presentation of the word *leather* in the second context (Context C) evokes the word from the first word pair. Proactive interference is strong with scopolamine. B: Left: With suppression of intrinsic synaptic transmission by acetylcholine (ACh; dotted line drawn through connections), retrieval of the first word pair is prevented, allowing more selective activity in response to the sensory input of the word *holster.* Middle: In this case, Hebbian synaptic modification strengthens only the desired connections within the network. Right: During retrieval, presentation of the word *leather* in the second behavioral context (Context C) can now evoke the word *holster.* Proactive interference is greatly reduced by acetylcholine.

impaired at acquiring overlapping than nonoverlapping odor pairs relative to controls. The design of the present study is summarized in Figure 3. A standard paired-associate paradigm was used for analyzing interference between encoded word pairs, in which subjects were trained and tested on one list of word pairs (A–B), and subsequently were retrained and tested on an overlapping list of word pairs (A–C) and a nonoverlapping list of word pairs (D–E). Previous studies have demonstrated proactive interference between lists, in which retrieval of the A–C pairs is decreased as a result of the prior encoding of the A–B pairs (Crowder, 1976). The computational modeling of cholinergic blockade suggests that scopolamine should enhance this proactive interference, thereby causing a larger impairment in recall of overlapping word pairs (A–C) than of nonoverlapping word pairs (D–E). We tested the hypotheses that:

1. Scopolamine would impair new learning of novel word-pair associates, but not those learned just prior to scopolamine administration.

2. Scopolamine would impair cued recall of overlapping word-pair associates more than that of nonoverlapping word-pair associates.

Method

Subjects and Study Groups

A total of 28 (9 men, 19 women) young healthy native-English speakers, with a mean age of 21 years (range = 18–29) and a mean education level of 14 years, participated in the study. All participants were recruited from a university community and were either in college or had a college degree.

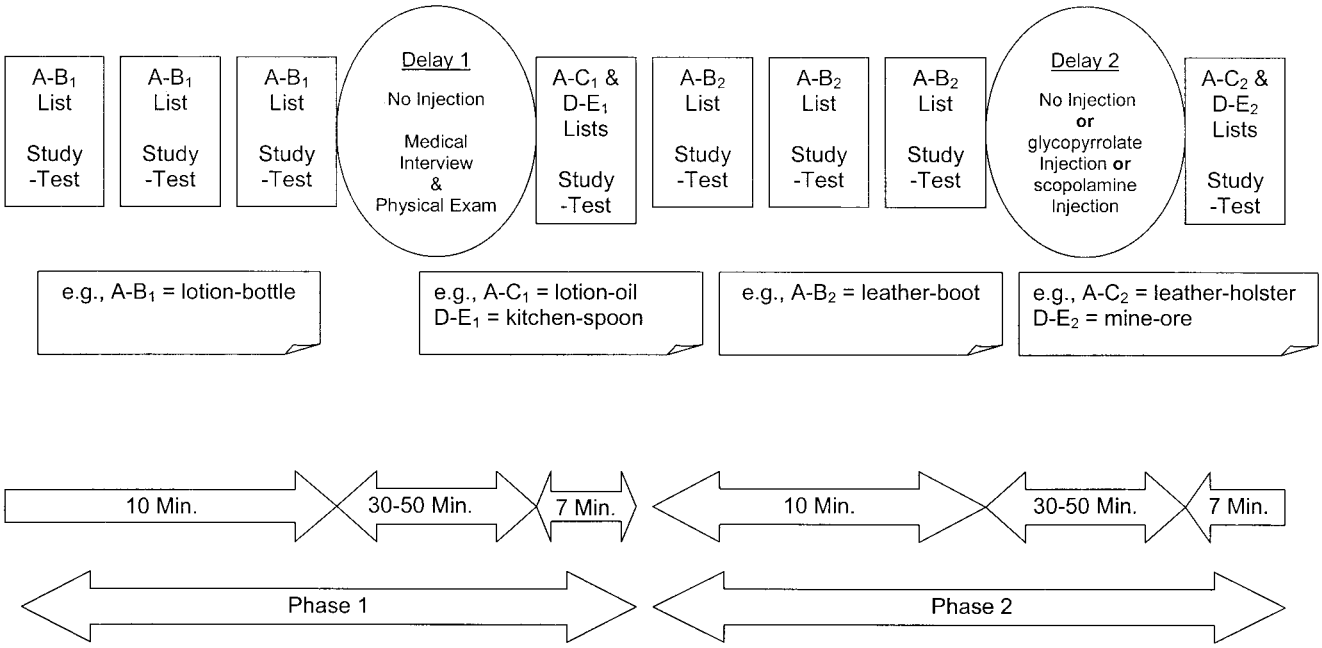

Figure 3. Design of the experiment. Subjects were tested on overlapping and nonoverlapping word pairs in two different phases of the experiment: Phase 1 and Phase 2. This allowed both a within-subject comparison of the effect of study phase and a between-groups comparison. Subjects were initially trained and tested three times on a list of 16 related pairs ($A–B_1$). After a delay, they were presented once with 16 overlapping pairs ($A–C_1$) and 16 nonoverlapping pairs ($D–E_1$) and tested on their cued recall of these new pairs. They were then trained and tested three times on a completely different set of paired associates ($A–B_2$). Then they were either injected with scopolamine or glycopyrrolate or not injected. After a delay, they were trained and tested on 16 overlapping and nonoverlapping pairs ($A–C_2$ and $D–E_2$). Theoretical considerations and model simulations predicted a larger difference between cued recall of $A–C_2$ and $A–C_1$ than between $D–E_2$ and $D–E_1$.

The study participants were divided into three groups: a no-drug group (n = 8, 3 men and 5 women), a glycopyrrolate group (n = 8, 1 man and 7 women), and a scopolamine group (n = 12, 5 men and 7 women). Informed consent was obtained in a manner approved by the Human Research Studies Committees of Massachusetts General Hospital and Boston University. All drug-injected subjects were screened for general medical conditions, especially those that could potentially be aggravated by administration of anti-cholinergic drugs. A physician took a detailed medical history and performed a medical and neurological examination. Female subjects were administered a urine pregnancy test immediately before drug injection. Subjects currently taking medications and those with histories of significant medical, neurological, or mental illness were not enrolled. Subjects were paid $50 for their participation.

Drug Administration, Monitoring, and Side Effects

Near the start of the delay period during Phase 1 of the experiment, all subjects, except those in the no-drug group, received an injection of either scopolamine or glycopyrrolate. Glycopyrrolate was administered at 4 μg/kg (preparation concentration of 0.2 mg/ml), and scopolamine hydrobromide (preparation concentration of 0.4 mg/ml) was administered at 8 μg/kg via intramuscular injection in the deltoid muscle. Similar dosages of scopolamine and glycopyrrolate have been used extensively in studies of scopolamine effects on human memory function (Beatty et al., 1986; Ghoneim & Mewaldt, 1975, 1977; Grober et al., 1989). The physician closely monitored subjects for drug side effects. Scopolamine significantly increased pupil size and produced transient dryness of the mouth, but did not have a significant effect on respiratory rate, heart rate, or blood pressure. Glycopyrrolate produced mild dryness of the mouth in half the subjects, but did not significantly affect other physiological measures. Subjects were escorted home after being observed for a minimum of 4 hr after drug administration and until it was deemed safe to do so.

Stimuli

The stimuli consisted of multiple lists of word pairs that, for purposes of illustration, fall generically into list categories: A–B, A–C, or D–E. Each word pair is either semantically related (e.g., robber–jail) or unrelated (e.g., stereo–fudge). For every related word pair in a list A–B, there is a corresponding word pair in list A–C that shares the same first (A) word but has a different second (C instead of B) word (e.g., A–B list paired association "robber–jail", and A–C list paired association "robber–police"). Therefore, lists A–B and A–C contain related word pairs and are also overlapping lists (i.e., the sets of pairs A–B and A–C intersect). However, lists A–B and D–E are nonoverlapping.

To control for specific effects of individual word pairs, pilot studies were performed to obtain lists that were relatively homogeneous in their ease of memorization. In addition, the entire study was counterbalanced with four lists of word pairs, such that each set of word pairs was utilized in each of the four experimental conditions (Phase 1–overlapping, Phase 1–nonoverlapping, Phase 2–overlapping, Phase 2–nonoverlapping). Each of the four permutations of list positions was utilized in 3 of the 12 test subjects in the scopolamine group and 2 of the 8 test subjects in the no-drug group and the glycopyrrolate group.

Behavioral Tasks

The format of the behavioral tasks consisted of a study period in which word pairs were presented on the computer screen, and a test period in which cued recall was tested in two different tasks: standard cued recall, and Modified Modified Free Recall (MMFR) (Crowder, 1976; Postman & Underwood, 1973).

Study

During the study period, participants were shown a list of word pairs presented simultaneously on a computer screen, and were asked to determine whether the words in each pair were related or unrelated. This low-level discrimination task was used to ensure that subjects would devote sufficient attention to the word pairs during their encoding. Responses were made manually by pressing the "R" (related) or "U" (unrelated) buttons on a standard computer keyboard. The stimulus (i.e., word) pairs were presented until subjects made a key press or until 1.5 s had elapsed. Subjects received immediate feedback as to whether or not their responses were correct. If the subject made a correct response within the time allotted, a high-pitched beep was presented. If an incorrect response was made or if the subject did not respond in the time allotted, a low-pitched beep of short duration was heard.

Test

During testing, cued recall of the second word of a paired-associate was tested in two procedures:

1. Standard cued recall in which the first word of a word pair was presented on the computer screen, and the participant was required to respond by typing the corresponding most recently presented second word of the paired association.

2. MMFR task in which the first word of an overlapping pair (e.g., robber) was presented on the computer screen and the participant was required to respond by typing the two words associated with it: one from list A–B (MMFR-B), and one from list A–C (MMFR-C; e.g., jail, police).

The MMFR procedure was used in addition to standard cued recall to avoid effects of response competition on retrieval.

Experiment Time Line

The overall experimental design and time line is summarized in Figure 3. The experiment focused on testing the encoding and cued recall of word paired associates. The full proactive interference design was performed twice in each subject, first in Phase 1 and then in Phase 2 of the experiment. During Delay 2 (part of Phase 2), the subjects were either injected with scopolamine or glycopyrrolate, or were not injected. This allowed for both a within-group comparison of performance on overlapping and nonoverlapping word pairs in Phases 1 and 2 of the study, and a between-groups comparison for the different groups (scopolamine, glycopyrrolate, no-drug).

In an initial practice phase of the experiment, subjects were given two practice study–test sequences with different word pairs in each. Subjects were trained on a list of 18 related paired associates (e.g., robber–jail) intermixed with 18 unrelated paired associates (e.g., stereo–fudge). Six additional paired associates were presented at the beginning and at the end of each training list to control for primacy and recency effects. At the end of each study period, subjects were tested on their ability to recall the second word of each related pair when cued with the first word (e.g., cue: robber, correct response: jail), initially by standard cued recall and, during a later test, by MMFR. They subsequently commenced Phases 1 and 2 of the experiment.

In Phase 1, subjects were engaged on a novel study–test sequence with a list of 18 related (list $A–B_1$) and 18 unrelated paired associates (with 6 additional paired associates at the beginning and at the end of the list). After each presentation of the list, they were tested on their ability to retrieve the related word pairs when cued with the A word. This study–test sequence was repeated two more times followed by a delay (Delay 1). The delay durations ranged between 30 and 50 min (due to slight variation in length of medical examinations and injection procedures), were held constant within subject for Phases 1 and 2 of the study, and were similar overall in the three groups. These time periods coincide with the time course for the onset of the amnestic side effects of scopolamine in young healthy subjects (Bishop, Curran, & Lader, 1996; Dundee & Pandit, 1972; Ebert, Siepmann, Oertel, Wesnes, & Kirch, 1998; Pandit & Dundee, 1970; Safer & Allen, 1971; Wesnes, Simpson, & Kidd, 1988). During the delay periods, subjects performed distractor tasks to avoid rehearsal of the word pairs. After Delay 1, subjects were trained on a mixed list of 36 new related word pairs drawn from two categories: 18 pairs that overlapped with the previously learned pairs (List $A–C_1$, e.g., robber–police), and 18 pairs that did not overlap with the previously learned pairs (List $D–E_1$). After one training session, their cued retrieval of the second word of all 36 of the related pairs was tested. This provided the primary comparison of performance on overlapping versus nonoverlapping word pairs. Subsequently, subjects performed the MMFR task.

After completion of Phase 1, Phase 2 of the study was initiated. First, the subjects repeated the study–test sequence three times with a new list of 18 related paired associates (List $A–B_2$) and 18 unrelated paired associates. Next, during Delay 2 (ranging between 30–50 min), they were either injected with scopolamine or glycopyrrolate, or were not injected. As in Delay 1, all subjects were again distracted to prevent rehearsal during Delay 2. Following Delay 2, subjects were shown a new mixed list of paired associates drawn from two categories: 18 pairs that overlapped with pairs from the list studied immediately before Delay 2 (List $A–C_2$), and 18 pairs that did not overlap (List $D–E_2$). As before, this list also contained 36 unrelated pairs. Finally, memory for the pairings was tested by the same cued retrieval tests used in Phase 1, including the MMFR test. The repetition of the full design allowed comparison of Phases 1 and 2 for both overlapping and nonoverlapping word pairs by within- and between-groups analyses.

Statistical Analysis

During testing, after each delay period, in response to the cue word (first word), subjects provided the second word of the most recently presented word pair. The number of correctly recalled second-position words was counted, with minor spelling errors counted as correct. The main analyses of the experimental data focused on comparing the differences between the groups in the number of correctly recalled words for (a) all word pairs between Phases 1 and 2, and (b) overlapping pairs versus nonoverlapping pairs between Phases 1 and 2. Following methods similar to DeRosa and Hasselmo (2000), we analyzed the data, in the context of the a priori hypotheses, for main effects by repeated measures analysis of variance (RM-ANOVA) and post hoc tests, and for specific interaction effects by focused contrast analysis.

Following the methods of Rosenthal, Rosnow, and Rubin (2000), we defined a linear contrast model according to our a priori hypotheses. The model was used in a focused analysis to assess the hypothesis that cued recall of overlapping word pairs encoded after scopolamine injection would be significantly lower than that of nonoverlapping word pairs. Our predictions were that all three groups would perform similarly in Phase 1, and that in Phase 2 scopolamine-injected subjects would perform worse than the other subjects, both overall and, particularly, in the recall of overlapping word pairs. We did not assume a priori that glycopyrrolate-injected subjects would show any memory deficits compared with noninjected subjects, and therefore, this was not built into the contrast analysis

Table 1
Mean (± SEM) Number of Words Correctly Recalled in the Different Study Conditions by Group, Word-Pair Task, and Study Phase

Group	$A–C_1$	$A–C_2$	$D–E_1$	$D–E_2$	MMFR-B_1	MMFR-B_2	MMFR-C_1	MMFR-C_2
Scopolamine	10.58 ± 0.85	6.50 ± 1.09	10.58 ± 0.79	8.33 ± 1.31	17.25 ± 0.31	17.58 ± 0.23	10.92 ± 0.91	6.17 ± 1.22
Glycopyrrolate	10.50 ± 1.13	8.50 ± 1.09	9.50 ± 0.91	9.75 ± 1.00	16.25 ± 0.70	16.38 ± 0.50	10.50 ± 1.28	8.88 ± 1.16
No-drug	11.50 ± 1.05	10.63 ± 1.22	11.50 ± 0.85	11.63 ± 1.48	16.63 ± 0.71	16.00 ± 1.05	11.38 ± 1.03	10.50 ± 1.35

Note. Letters A–E represent the different word lists. Subscript numbers represent study phases. MMFR = Modified Modified Free Recall.

(i.e., we assume in the model that glycopyrrolate-injected and noninjected subjects will perform equally well). The equations for the contrast analysis can be found in the Appendix. Statistical analyses were performed with the programs Microsoft Excel, StatView, and SAS (SAS Institute, Cary, NC).

Results

The summary statistics of the raw data are presented in Table 1.

Effects on Word Paired-Associate Learning

Results show a main effect of scopolamine on word paired-associate learning, $F(2, 25) = 3.74$, $p = .04$, with subjects showing a decrease in cued recall of the second word of all paired associates after scopolamine compared with the glycopyrrolate and the no-drug groups. This effect can be seen in Figure 4 as the difference in the mean number of words recalled in both word type categories in the scopolamine group (Figure 4A) compared with the glycopyrrolate (Figure 4B) and no-drug (Figure 4C) groups. There is a clear decrease in recall of both categories of word pairs after encoding under scopolamine.

Effects on Learning Overlapping Versus Nonoverlapping Pairs

A focused contrast analysis was used to decompose the a priori hypothesized interaction of Group × Phase × Word-Pair Type (i.e., interaction of Scopolamine × Phase 2 × Overlapping Word Pairs). We predicted that scopolamine injection would cause a greater impairment of the encoding of overlapping word pairs relative to that of nonoverlapping word pairs. The focused contrast yielded significant results, $F(1, 73) = 13.10$, $p < .01$. In other words, there was a significant specific decrease in the cued recall of overlapping word pairs in scopolamine-injected subjects, compared with those injected with glycopyrrolate and those not injected, and with the cued recall of nonoverlapping word pairs. This interaction effect can be seen in Figure 5, comparing overlapping (A–C) pairs in Figure 5A with nonoverlapping pairs (D–E) in Figure 5B. Analysis of the recall of overlapping pairs (A–C) versus nonoverlapping pairs (D–E) also demonstrated an overall significantly better encoding of nonoverlapping word pairs in Phase 2 regardless of group, $F(1, 25) = 5.11$, $p = .03$.

Effects on the MMFR Task

The MMFR task allows testing of retrieval of both A–B pairs and A–C pairs after the delay period in both phases 1 and 2 of the study for different groups. Scopolamine did not reduce the retrieval of the A–B pairs (Figure 6A). The mean (± *SEM*) A–B retrieval before scopolamine injection (in Phase 1) was 17.25 ± 0.30; and after scopolamine injection (in Phase 2), 17.58 ± 0.23. The combined mean cued recall for the no-drug and glycopyrrolate groups was 16.48 ± 0.48 in Phase 1 and 16.19 ± 0.56 in Phase 2.

The MMFR task also allows testing of A–C retrieval in the absence of direct competition from A–B retrieval. Retrieval of both words without regard to the order in which they were presented will prevent confusion about which word was encoded after the delay, preventing interference caused by response competition. Despite this lack of competition during retrieval, there was still a significant interaction of Group × Phase × Word Pair Retrieval Task, $F(2, 25) = 5.63$, $p = .01$, for decreased performance on MMFR-C after scopolamine injection (i.e., Scopolamine × Phase 2 × MMFR-C interaction effect) compared with the other five conditions. This effect can be seen in Figure 6B.

Types of Errors

Most of the errors occurring during cued recall for overlapping pairs involved nonresponses (i.e., blank responses) rather than direct intrusions. The difference in the mean number of times the subjects did not provide a cued response (i.e., number of blanks) from Phase 1 to Phase 2 was significantly higher in the scopolamine group compared with the no-drug and glycopyrrolate groups, $F(2, 25) = 4.97$, $p = .02$. In other words, there was a significant Group × Phase (i.e., Scopolamine × Phase 2) interaction for nonresponses.

Overall, there was a trend for more intrusions from the B-list during cued retrieval of the C-list compared to intrusions from other lists already presented ($F = 4.04$, $p = .06$; i.e., main effect of B-list intrusions). However, there was a significant increase in the probability[1] of having an intrusion from the B-list in Phase 2 of the experiment during C-list retrieval ($F = 5.68$, $p = .03$). Specifically, the probability of having a B-list intrusion during C-list cued recall after scopolamine injection was .157, whereas that of the other five conditions ranged from .021 to .086.

The participants also made significantly more semantically related than semantically unrelated errors when responding incorrectly with a word not previously presented in the experiment ($F = 58.20$, $p < .01$). However, RM-ANOVA did not reveal a statistically significant interaction of drug group and experimental phase

[1] The intrusion rate for each intrusion type was calculated based on the number of observed intrusions of that type (the numerator) divided by the number of items that had been presented at each previous stage of the study that could potentially serve as an intrusive item (the denominator). The denominators for B-list intrusions in Phase 1 and Phase 2 were 18 and 96, respectively, while those for Other-list intrusions were 18 and 264, respectively.

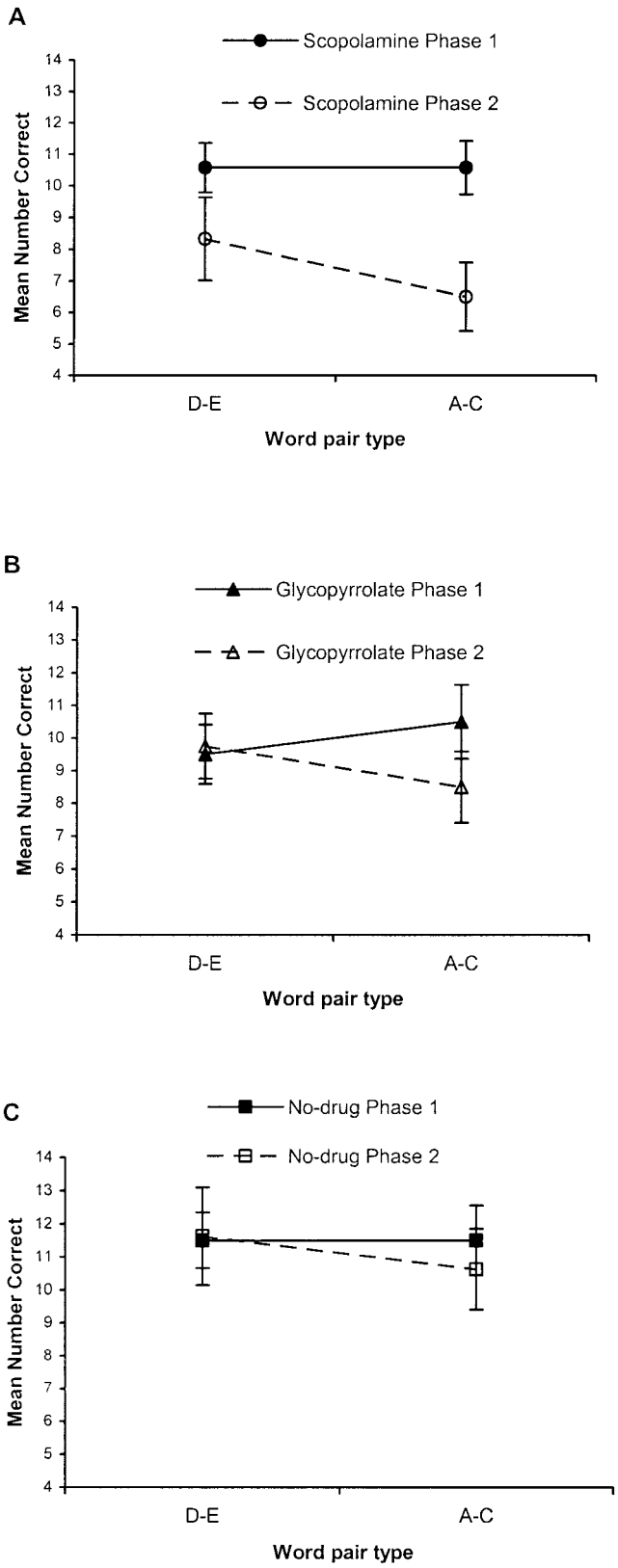

Figure 4. Performance on cued recall of overlapping (A–C) and nonoverlapping (D–E) word pairs in the different groups. A: Mean number of words correctly recalled before (Phase 1) and after (Phase 2) scopolamine injection. There is a significant decrease in cued recall of both overlapping and nonoverlapping word pairs after scopolamine injection, with a greater impairment in the recall of overlapping word pairs. B: Mean number of words correctly recalled before (Phase 1) and after (Phase 2) glycopyrrolate injection. Though fewer overlapping word pairs were recalled after glycopyrrolate injection, this trend was not statistically significant. C: Mean number of words correctly recalled before (Phase 1) and after (Phase 2) Delay 2 in subjects in the no-drug group. No-drug group subjects recalled overlapping and nonoverlapping word pairs equally well in both phases of the study. All plotted values are means (± 1 *SEM*) and are provided in Table 1. Perfect recall would be 18.

($F = 0.79$). The number of spelling errors was not significantly different in any specific group or phase ($F = 0.22$).

Discussion

The data presented here provide evidence that blockade of central muscarinic cholinergic receptors by scopolamine impairs encoding of word paired associates, and that cued recall of overlapping word pairs is more affected than that of nonoverlapping word pairs. Interpreted in the context of previous theoretical and computational models (Hasselmo & Wyble, 1997), and evidence from the animal and human memory literature, these results support the conclusion that acetylcholine plays an important role in associative learning by decreasing proactive interference.

The results presented here suggest that scopolamine decreases the learning of new word paired associates, but does not negatively affect the retrieval of previously learned novel word pairs. Subjects showed statistically significant decreases in cued recall for the second word of all paired associates learned after scopolamine injection (i.e., a relative decline in performance in Phase 2 compared with Phase 1 on the A–C, D–E, and MMFR-C tasks) when compared with those after glycopyrrolate injection or no injection. However, subjects did not show such recall deficits for paired associates (A–B pairs) that were well-learned before scopolamine injection (i.e., overall performance on the MMFR-B task was relatively stable across Phase 1 and Phase 2 of the study). This suggests that the effects of scopolamine on cued recall of other word pairs were due to the presence of scopolamine during encoding, rather than a nonspecific effect on retrieval capacities.

The data also support the prediction that scopolamine should increase proactive interference and, thus, more strongly impair the encoding of overlapping than nonoverlapping word pairs. This interaction can be seen in Figures 4, 5, and 6B when one compares performance on A–C and MMFR-C to D–E after scopolamine injection, and also when this comparison is made in the other groups. Intrusions from the B list (the second word of the A–B word pair) may be viewed as an indirect measure of proactive interference from a previously well-learned word list. The B-list intrusion rate after scopolamine injection was 0.157, which is 2–8 times higher than the rate in the other study conditions.

The effects of cholinergic blockade shown in this study are consistent with the effects of acetylcholine in cortical structures, and with predictions from computational models that suggest that cholinergic blockade should enhance proactive interference (Hasselmo & Bower, 1993; Hasselmo et al., 1996; Hasselmo & Wyble, 1997). This increase in proactive interference could result from

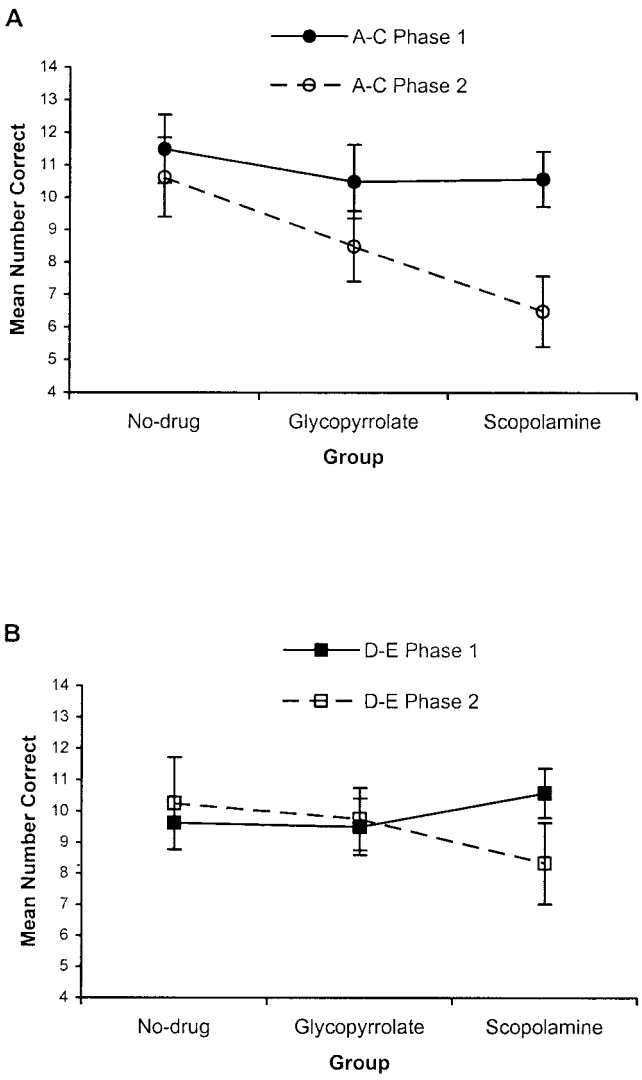

Figure 5. Performance on cued recall in the different groups by word-pair type. Data in Figure 4 are presented differently to show side-by-side performance in the different groups (no-drug, glycopyrrolate, scopolamine) on each word pair type separately. A: Mean number of overlapping (A–C) words correctly recalled in the different groups separated by study phase. Overall, fewer overlapping words were recalled in Phase 2 in all groups, there was a linear trend for worse recall going from the no-drug group to the glycopyrrolate group to the scopolamine group, and scopolamine-injected subjects recalled significantly fewer overlapping words. B: Mean number of nonoverlapping (D–E) words correctly recalled in the different groups separated by study phase. Recall of nonoverlapping words was significantly diminished after scopolamine injection (Scopolamine Phase 2), whereas noninjected and glycopyrrolate-injected subjects performed equally well in both phases of the study. All plotted values are means (± 1 *SEM*) and are provided in Table 1. Perfect recall would be 18.

blockade of the cholinergic suppression of excitatory synaptic transmission (Hasselmo & Schnell, 1994; Hounsgaard, 1978). This decrease in cholinergic suppression would allow for the spread of excitatory activity across previously modified synapses to interfere with encoding of new overlapping word pairs, as illustrated in Figure 2. As shown in this figure, the increase in proactive interference could be manifested as either an increase in erroneous intrusions from the initially learned pair (A–B) or simply as a decreased retrieval output of the newer paired associate (A–C). The analysis of errors in the present study shows a statistically significant main effect of scopolamine injection on nonresponses (i.e., blank responses) and a trend for an increased rate of B-list intrusions.

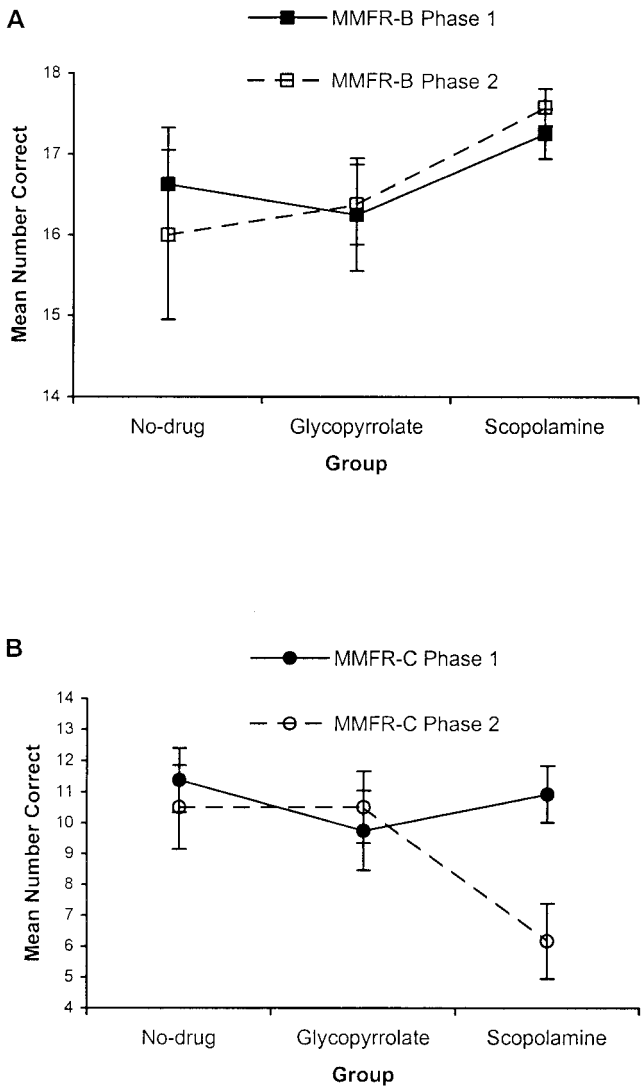

Figure 6. Side-by-side performance in the different groups (no-drug, glycopyrrolate, scopolamine) shown separately on each Modified Modified Free Recall task (MMFR-B and MMFR-C). These data suggest that scopolamine affected new learning and not retrieval processes. A: Mean number of words correctly recalled on the MMFR-B (i.e., B words recalled when cued with the corresponding A words) in the different groups separated by study phase. There was no decrease in performance on MMFR-B after scopolamine. B: Mean number of words correctly recalled on the MMFR-C (i.e., C words recalled when cued with the corresponding A words) in the different groups separated by study phase. Performance on MMFR-C was significantly diminished only after scopolamine injection (Scopolamine Phase 2). All plotted values are means (± 1 *SEM*) and are provided in Table 1. Perfect recall would be 18.

The increased number of nonresponses on the A–C, D–E, and MMFR-C tasks are unlikely to be due to less specific global effects such as inattention, lack of effort, or susceptibility to glare from the computer screen under scopolamine, as these would be expected to also detrimentally affect performance on the MMFR-B task. However, as seen in Figure 6A, performance on MMFR-B was the same or better after scopolamine injection, especially when compared with the performance of the no-drug and glycopyrrolate groups in Phase 2. It is important to observe that Troster et al. (1989) also noted that subjects given 0.5 mg scopolamine had a slightly higher overall performance on recall of remote semantic information than when they were injected with either saline or higher doses of scopolamine (0.8 mg). As previously noted, the B-list intrusion rate after scopolamine injection was 2–8 times higher than this rate in the other study conditions, but we could not justify performing a post hoc focused contrast analysis, as this was not a specific a priori hypothesis we had set out to demonstrate in this study. Although significant levels of prior-item and extra-list intrusions were not seen in previous studies using scopolamine in short-term memory tasks such as the Brown-Peterson paradigm (Beatty et al., 1986; Troster et al., 1989), Frith et al. (1984) found that scopolamine increased current-list intrusion errors during immediate serial free recall of verbal items. Though they tested a different memory process, short-term list item memory, compared to our study of long-term memory for novel paired associates, it is nonetheless interesting to note that Beatty et al. (1986) and Troster et al. (1989) both observed that the rates of extra-list intrusions (but not prior-item intrusions), though not reaching statistical significance, were at least 2 times greater, and the percentage of perseverative responses were always higher in the Brown-Peterson paradigm under scopolamine.

Although one may postulate "task difficulty" (the most difficult memory condition being the most susceptible to drug effects) as an alternative explanation to the effects of scopolamine on performance on the A–C task, this provides a less likely scenario in the face of the overall data. The task difficulty explanation relies on the facts that performance in this study on the A–C task declined across time (see Figure 4), that this is one of the high-interference conditions, and that it is the most difficult memory condition. What argues against this explanation is that performance on the D–E task did not degrade nearly as much under scopolamine. One may plausibly argue that the D–E task is equally, if not more, difficult a task under standard conditions as the A–C task. In the D–E task one is required to learn unfamiliar, individual words in pairs under temporal constraints such that engaging cognitive strategies would be difficult, therefore imposing a great memory demand on the subject. In fact, performance on the D–E task in Phase 1, when summed over all groups, was marginally worse than performance on the A–C task, arguing that D–E is at least as difficult a memory condition as A–C.

Although there was a significant decrease in cued recall of all word pairs after scopolamine injection, this contrasted with a complete absence of effects on cued recall of word pairs that were encoded before administration of scopolamine. This selective effect on encoding is consistent with evidence demonstrating that acetylcholine enhances modification of excitatory synapses in cortical structures, and that infusion of scopolamine prevents this cholinergic enhancement of long-term potentiation (Blitzer et al., 1990; Huerta & Lisman, 1993; Patil et al., 1998). Reduction of long-term potentiation will reduce the size of synaptic potentials induced by these modified synapses during retrieval, and thereby reduce the number of accurately retrieved associations. A decrease in the amount of synaptic modification would result in the selective effect of scopolamine on free recall of single words while having a weaker effect on the recognition of these words (Ghoneim & Mewaldt, 1975, 1977). Simulations have demonstrated how moderate decreases in synaptic modification could have a stronger effect on free recall versus recognition (Hasselmo & Wyble, 1997). Alternately, the dissociation between recall and recognition observed by Ghoneim and Mewaldt (1975) could reflect differential effects of cholinergic blockade on the hippocampus versus surrounding neocortical structures (e.g., perirhinal cortex; Norman & O'Reilly, 2003). Functional magnetic resonance imagery (fMRI) studies have supported an important role of the hippocampus in encoding and subsequent memory of new information in both the visual and verbal domains (Brewer, Zhao, Glover, & Gabrieli, 1998; Kirchhoff, Wagner, Maril, & Stern, 2000; Stern et al., 1996; Wagner et al., 1998).

Relatively few functional neuroimaging studies have examined the effects of cholinergic modulation on encoding (Grasby et al., 1995; Rombouts, Barkhof, Van Meel, & Scheltens, 2002; Sperling et al., 2002). To our knowledge, the only fMRI study that has assessed the effects of cholinergic blockade on the encoding of novel paired associates was by Sperling et al. (2002), who showed that there were significant decreases in activation within the hippocampal, fusiform, dorsolateral prefrontal, and inferior frontal regions under scopolamine during the encoding of novel face–name pairs. Impaired performance on post-scan recognition tests in their study inversely correlated with the extent and magnitude of blood oxygenation level-dependent (BOLD) signal activations during encoding. Grasby et al. (1995) used positron emission tomography (PET) to show that scopolamine attenuated auditory verbal memory-task-induced increases of regional cerebral blood flow (rCBF) in the left and right prefrontal cortices and the right anterior cingulate region. Meanwhile, Rombouts et al. (2002) recently used fMRI in patients with mild Alzheimer's disease (AD) who were given a single dose of rivastigmine, a cholinesterase inhibitor used to transiently increase synaptic acetylcholine levels, to show that BOLD signal activations were increased in the fusiform regions during encoding of novel faces. Though selective lesions of the hippocampus have been shown to impair paired-associate learning (Zola-Morgan, Squire, & Amaral, 1986), cholinergic blockade may, however, also produce interference effects in neocortical structures, including the frontal cortices. The frontal cortices have been implicated in preventing excessive proactive interference (Troyer, Winocur, Craik, & Moscovitch, 1999), and have found to be functionally affected by cholinergic modulation during memory tasks (Grasby et al., 1995; Sperling et al., 2002).

Though the cortical functional dynamics underlying recognition memory have not been completely elucidated in humans, some have postulated that the perirhinal cortex can support some degree of recognition memory on its own (Aggleton & Brown, 1999; Davachi, Mitchell, & Wagner, 2003). If cholinergic blockade affects cortical regions differently, then one would expect to find differential effects on encoding and recognition under scopolamine. Two recent studies have shown that scopolamine impairs item recognition in both the verbal (Mintzer & Griffiths, 2003) and visual domains (Sherman et al., 2003). We showed that scopol-

amine impairs visual item recognition memory and suggested, according to a quantitative dual-process recognition memory model, that scopolamine does not significantly affect the frequency with which recollection judgments are made, but rather that it distorts the quality of the recollection judgments (Sherman et al., 2003). Perhaps this type of distortion in the quality of recollection may have also contributed to the relative differences in recall performance in the A–C versus the MMFR-C tasks, and for the pattern of errors, especially intrusions, observed under scopolamine in the present study.

Considerable controversy has arisen around the potential role of cholinergic modulation in memory function versus attention. The hypothesis guiding the research in this paper is neutral with respect to this controversy, as it is based on cellular level effects of acetylcholine. It is, however, interesting to note that Curran, Pooviboonsuk, Dalton, and Lader (1998) used event-related potentials to conclude that although benzodiazepines and anticholinergic drugs, such as scopolamine, reduce arousal and induce amnesia, these effects are not interdependent, and that there is a dissociation between their effects on episodic memory and on arousal.

Relation to the Animal Literature

The results of this study are highly consistent with data on cholinergic effects in the animal literature. Most notably, these results extend to humans the findings and conclusions of DeRosa and Hasselmo (2000). They studied the effects of scopolamine on the encoding of overlapping versus nonoverlapping odor pairs in rats and showed that injections of scopolamine caused a greater impairment of discrimination of overlapping relative to nonoverlapping odor pairs. They concluded that, in rats, cholinergic blockade increases proactive interference between stored odor memories during associative learning. Interference effects could also contribute to the effects of muscarinic receptor blockade on the ability to learn a new platform location each day in the Morris water maze (Sutherland et al., 1982; Whishaw, 1985), and to the fact that systemic injections of scopolamine impair arm choice behavior in an eight-arm radial maze in rats when there is a delay between the individual choices (Bolhuis, Strijkstra, & Kramers, 1988). In monkeys, scopolamine impairs performance at longer delays in delayed matching-to-sample (DMS) tasks using simple repetitively presented stimuli, while sparing performance at 0-s delay (Bartus & Johnson, 1976; Penetar & McDonough, 1983). Even greater interference effects occur in delayed matching-to-place tasks in which only two places are utilized (Dunnett et al., 1990). Dose-dependent and delay-dependent impairments on a DMS task have also been demonstrated in humans injected with scopolamine (Robbins et al., 1997). Performance in these DMS tasks may depend on cholinergic modulation of cation currents that modulate sustained spiking activity in cortical structures, as supported by the cholinergic modulation of intrinsic spiking activity in slice preparations of entorhinal cortex (Egorov, Hamam, Fransen, Hasselmo, & Alonso, 2002; Klink & Alonso, 1997) and simulations of the behavioral effect in DMS tasks (Fransen, Alonso, & Hasselmo, 2002). These tasks show impairments with both systemic and localized injections of scopolamine (Dudchenko & Sarter, 1992; Dunnett et al. 1990; Ragozzino & Kesner, 1998; Shannon, Bemis, Hendrix, & Ward, 1990), and with medial septal lesions (Numan, Feloney, Pham, & Tieber, 1995; Numan & Quaranta, 1990), which destroy the cholinergic innervation of the hippocampal formation. Medial septal lesions also greatly impair reversal performance (M'Harzi et al., 1987). These animal studies suggest that acetylcholine levels normally play an important role in preventing previously established memories from interfering with the acquisition of new memories.

Relation to Memory Pathology in Humans

Increased proactive interference on memory tasks has been shown in conditions that result in cortical cholinergic denervation, including those seen in patients suffering from Korsakoff's syndrome and in patients with rupture of the anterior communicating artery that resulted in cerebral infarction (Cermak & Butters, 1972; Damasio, Eslinger, Damasio, & Kassell, 1985; Kinsbourne & Winocur, 1980; Mayes, Pickering, & Fairbairn, 1987; Vilkki, 1985). Cholinergic deficits have also been observed in a number of neurological conditions including AD, dementia with Lewy bodies (DLB), Parkinson's disease (PD), Huntington disease, and vascular–ischemic dementia (VaD; Cummings, 2000; Korczyn, 2001; Pompeu & Growdon, 2002; Postle, Locascio, Corkin, & Growdon, 1997). Although the typical pattern of cognitive and behavioral deficits varies greatly between these disorders, it remains plausible that the misattribution of episodic events and facts, hallucinations, and delusions in these diverse disorders may be, at least partially, due to a cholinergic deficit. It has been shown that increasing cerebral synaptic acetylcholine levels by means of acetylcholinesterase inhibitors can improve attention, memory, activities of daily living, and behavioral symptoms in patients with AD, DLB, PD, and VaD (Bullock & Cameron, 2002; Erkinjuntti et al., 2002; McKeith et al., 2000; Rogers, Farlow, Doody, Mohs, & Friedhoff, 1998; Trinh, Hoblyn, Mohanty, & Yaffe, 2003). Proactive interference observed with scopolamine in this study may suggest a mechanism that could contribute to the mnemonic and behavioral features of pathological conditions influencing cortical cholinergic innervation.

Trends in the data, interpreted in the context of previous evidence, suggest that glycopyrrolate, a predominantly peripherally acting anticholinergic, may produce subtle but measurable negative effects on memory and learning. Although all groups performed similarly in Phase 1 of the study, the performance of the glycopyrrolate-injected subjects in Phase 2 of the study consistently fell between that of the no-drug and scopolamine groups. Glycopyrrolate, a quaternary amine with predominantly peripheral anticholinergic effects, is often mistakenly thought to be solely a peripheral anticholinergic that "does not enter the central nervous system" (Grober et al., 1989). Though glycopyrrolate crosses the blood–brain barrier slowly and less well relative to the tertiary amines scopolamine and atropine, with time, sufficient amounts cross the blood–brain barrier in humans and in animals (Ali-Melkkila et al., 1993; McEvoy, 2002; Physician's Drug Reference, 2002). It is also known that glycopyrrolate may cause confusion and delirium in the elderly (McEvoy, 2002; Physician's Desk Reference, 2002). One study found that glycopyrrolate, when given to a geriatric population as a preanesthesic, exacerbated the confusion, amnesia, and delirium caused by electroconvulsive therapy, as much as atropine (Sommer, Satlin, Friedman, & Cole, 1989). Glycopyrrolate has also been shown to decrease the per-

ception of vertigo in Meniere's patients (Storper, Spitzer, & Scanlan, 1998). These are all consistent with glycopyrrolate exerting a central behavioral effect. We interpret the results in the present study as suggesting that scopolamine exerted both central and peripheral anticholinergic effects, and that while glycopyrrolate mimicked scopolamine's peripheral effects, it also exerted a fraction of its central effects. However, for the purpose of the focused contrast analysis, we adhered to our a priori hypothesis that only the performance of subjects in the scopolamine group would be negatively affected.

Some behavioral studies have used glycopyrrolate as an active placebo, without postulating a significant behavioral effect (Beatty et al., 1986; Grober et al., 1989). These negative findings may be due to the paradigms, doses, tasks, and methods of statistical analysis used in the studies. Perhaps with higher doses and with tasks requiring greater cognitive demands, such as the one in the present study, subtle behavioral trends may become clear. If glycopyrrolate exerts subtle behavioral effects, then studies that use this either solely or in combination as a control group may decrease their power to detect any differences between control and scopolamine-injected groups. This may be one factor that has contributed to the apparently inconsistent findings between studies that have and those that have not used active placebos in their control groups when studying the effects of scopolamine on attention, working memory, and item and associative memory (Beatty et al., 1986; Caine et al. 1981; Crow & Grove-White, 1973; Ghoneim & Mewaldt, 1977; Grober et al., 1989; Mintzer & Griffiths, 2001, 2003; Robbins et al., 1997; Safer & Allen, 1971; Sherman et al., 2003). Scopolamine injection has been found to significantly impair paired-associate memory for free recall of number–color associations (Crow & Grove-White, 1973), and cued recall of name in face–name associations (Sperling et al., 2002), but not for cued recall of digit in symbol–digit associations (Beatty et al., 1986), cued recall of self-generated (i.e., generated by free association with a given cue) and well-rehearsed semantic and phonemic word paired associates (Caine et al., 1981), or cued recall of colored pattern in colored pattern–spatial location associations (Robbins, et al., 1997). However, it is important to note that both Beatty et al. (1986) and Caine et al. (1981) combined active placebo (glycopyrrolate or methylscopolamine) and inactive placebo (saline) groups to obtain their control groups used for comparison to their scopolamine groups. Also, Robbins et al. (1997) suggest that the lack of scopolamine effect seen in the performance of their colored pattern–spatial location paired-associate task may have been due to the relatively low-demand nature of their task, particularly when performance on a modified version of the task that made it more cognitively demanding showed that scopolamine significantly affected performance. Of greatest interest to the present study is the observation that discrimination of overlapping odors by rats in the study of DeRosa and Hasselmo (2000) consistently showed the same patterns of performance across various dosage ranges: Rats performed at an intermediate level when injected with methylscopolamine compared with when they were injected with saline (best performance) or with scopolamine (worst performance). A similar pattern was noted for performance of glycopyrrolate-injected subjects in the present study. For this particular rank ordering of performance to occur consistently in a number of similar tasks across dosages and species is highly unlikely to be due to chance alone. Therefore, if glycopyrrolate or methylscopolamine exert subtle but measurable behavioral effects when they are used as an active placebo and their results are combined with a passive placebo group to serve as a combined control group versus a scopolamine group, then one would expect a decrease in power for detecting the behavioral effects of scopolamine in these studies.

In summary, the experimental results presented here provide strong support that the centrally acting muscarinic antagonist scopolamine impairs learning of new word paired associates, and that the encoding of overlapping word pairs is more affected than the encoding of nonoverlapping word pairs. These results are consistent with computational models and neurophysiological and neuropathological data from humans and animals which suggest that, during new learning, cerebral hypocholinergic states may enhance proactive interference effects stemming from previously encoded and associated stimuli.

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Appendix

Contrast Analysis

Contrast (L) scores for individuals were calculated by summing the products of their scores (Y_i) with the corresponding model weights (λ_i), and an F score, $F_{contrast}$, was calculated according to the following equation:

$$L = \sum_i \lambda_i Y_i$$

$$F_{contrast} = \frac{MS_{contrast}}{MS_{within}}$$

$$MS_{contrast} = \frac{n_h L^2}{\sum_i \lambda_i^2}$$

$$MS_{within} = \frac{\sum_i (n_i - 1)\, S_i^2}{df_{within}}$$

$$df_{within} = \sum_i (n_i - 1)$$

where $MS_{contrast}$ is the mean square (i.e., sum of squares) attributable to the contrasts (L scores), MS_{within} is the pooled estimate of the population variance for the L scores, n_h is the harmonic mean (which is used because the groups sizes are unequal), n_i is the number of subjects in the i-th group, S_i^2 is the unbiased estimate of variance in the i-th group, and df_{within} is the degrees of freedom within groups (Rosenthal et al., 2000).

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