

# Muscarinic Cholinergic Neuromodulation Reduces Proactive Interference Between Stored Odor Memories During Associative Learning in Rats

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Previous electrophysiological studies and computational modeling suggest the hypothesis that cholinergic neuromodulation may reduce olfactory associative interference during learning (M. E. Hasselmo, B. P. Anderson, & J. M. Bower, 1992; M. E. Hasselmo & J. M. Bower, 1993). These results provide behavioral evidence supporting this hypothesis. A simultaneous discrimination task required learning a baseline odor pair (A+B-) and then, under the influence of scopolamine, a novel odor pair (A-C+) with an overlapping component (A) versus a novel odor pair (D+E-) with no overlapping component. As predicted by the model, rats that received scopolamine (0.50 and 0.25 mg/kg) were more impaired at acquiring overlapping than nonoverlapping odor pairs relative to their performance under normal saline or methylscopolamine. These results support the prediction that the physiological effects of acetylcholine can reduce interference between stored odor memories during associative learning.

One of the major problems in understanding memory concerns how similar items are stored and retrieved so that the individual identity of the items is preserved without interference. We are specifically interested in proactive interference, which occurs when previously learned information impairs the acquisition of new, related information.

A considerable body of research has implicated acetylcholine (ACh) as an important neurotransmitter in learning and memory processes, but there is disagreement about the specific role the cholinergic system plays in learning and memory (Blokland, 1996; Hunter & Murray, 1989). A dense population of cholinergic neurons in the central nervous system can be found distributed across several nuclei in the basal forebrain, which has widespread telencephalic projections including the hippocampus, olfactory bulb, and olfactory cortex (Renner, Dodson, & Leduc, 1992). An advantage of using odor stimuli to study memory is that the olfactory cortex is monosynaptically connected to areas implicated in memory formation, such as the hippocampus and frontal cortex (Lynch & Staubli, 1993; Slotnick & Risser, 1990; Staubli, Fraser, Faraday, & Lynch, 1987).

Muscarinic cholinergic transmission, specifically, has been shown to be required for performance in an olfactory

delayed match-to-sample discrimination task, as supported by the fact that scopolamine impaired performance on this task in a dose- and time-dependent manner (Ravel, Vigouroux, Elaagouby, & Gervais, 1992). Scopolamine and bilateral radio frequency lesions of the horizontal limb of the diagonal band of Broca also impaired delayed matching in a two-odor, olfactory successive discrimination task (Roman, Simonetto, & Soumireu-Mourat, 1993). In this task, the lesioned rats could not make the odor-reward association consistently with a delay longer than 15 s.

In olfactory habituation tasks, adult rats repeatedly exposed to the same conspecific decrease their olfactory social investigation of that conspecific. Scopolamine disrupts this habituation and increases the social olfactory investigation of familiar juvenile conspecifics by adult rats (Soffie & Lamberty, 1988). Although rats habituate to an odor after repeated exposure, they are still able to discriminate and respond to a second, novel odor stimulus. Rats treated with scopolamine failed to show both habituation to a familiar odor and recognition of a novel odor (Hunter & Murray, 1989). This effect was not mediated by the hippocampus and was demonstrated in rats that had received electrolytic lesions to the medial septum. These septal-lesioned rats continued to habituate to familiar odors and respond to novel odors.

In our laboratory, electrophysiological studies in the olfactory cortex have demonstrated that ACh and cholinergic agonists strongly suppress transmission at the intrinsic fibers between pyramidal cells but have little effect on the synaptic transmission at the afferent fibers originating from the lateral olfactory tract (Hasselmo & Bower, 1992; Linster, Wyble, & Hasselmo, 1999). Cholinergic agonists have been shown to suppress adaptation of pyramidal cell firing in brain slices of the olfactory cortex (Barkai & Hasselmo, 1994) and enhance the relative amplitude of long-term

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This work was supported by a Harvard University Sackler Prize Fellowship in Psychobiology to Eve De Rosa and National Science Foundation Grant IBN 9996177. We thank Ross Bergman and Tim Otto for their technical assistance on the olfactometer as well as Christiane Linster and Mark Baxter for their constructive comments on an earlier version of this article.

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potentiation in the olfactory cortex (Hasselmo & Barkai, 1995; Patil, Linster, Lubenov, & Hasselmo, 1998). A computational model, based on this physiological evidence, suggests that when ACh induces these effects on the cortex, it may act to reduce interference between associated items stored in memory (Hasselmo, Anderson, & Bower, 1992).

According to the model, the loss of cholinergic modulation in the olfactory cortex leads to excitatory spread across previously modified intrinsic connections (Hasselmo & Bower, 1993; Hasselmo et al., 1992). This effect allows previously learned patterns to interfere during the learning of new patterns. This prediction guided our behavioral hypothesis that learning odor associations with elements that overlap with previously learned odor associations would produce more errors than would acquiring nonoverlapping odor associations when the rat is under the influence of scopolamine.

Using our A+B-, A-C+ paradigm, we predicted that inappropriate responses would appear in the critical overlapping task. The baseline condition required the rats to learn odor pair A+B-. When A occurred again in the new overlapping odor pair (A-C+), a response of A instead of C would initially be elicited and would compete with the response to C for actual emission. Associative inhibition of C, because the rats previously learned A+, would make C more difficult to learn (see Figure 1). Interference is thought to arise when the original and second odor pair share some common stimulus element (e.g., A) but require different responses (A+B- vs. A-C+). Thus A appears more often because it is necessary as a source of proactive interference.

Our primary assumption is that the processing necessary for this task is taking place in the olfactory cortex. The cholinergic effects hypothesized to prevent proactive interference in the olfactory cortex could also be at work in other cortical structures, including the hippocampus; although the general representation of stimuli may be quite different. A similar laminar selectivity of cholinergic suppression has been demonstrated in the hippocampal formation (Hasselmo & Schnell, 1994). Specifically, synaptic modification of the Schaffer collaterals may store associations between activity in region CA3 and the afferent input to region CA1 from the entorhinal cortex. Thus, ACh may have similar functional significance in different brain regions.

The firing properties of single pyramidal neurons were measured in the olfactory cortex in behaving rats performing in a successive-cue, olfactory discrimination task (Schoenbaum & Eichenbaum, 1995). Neural activity occurred during the following trial elements: (a) initiating a trial with a nose poke, (b) sampling the odor stimuli, and (c) responding to a trial by nose poking the water port. The olfactory cortical neurons encoded the odor stimuli characteristics during sampling and maintained a representation of the odor stimuli during the response. Associations between the neural activity encoding different task components in the olfactory cortex could underlie learning of the task utilized here (see Figure 1). In our task, the rats were required to learn the following three-term contingency: (a) the discriminative stimulus that precedes the response (odor present); (b) the response itself (nose poke), and (c) the stimuli that follow

the response, (e.g., the reinforcer that occurred only after a correct operant response and the red light that occurred after an incorrect operant response). The task demanded that the rat form an association of the odor pairs with a particular response rather than withholding a response to a single odor, as required in a go no-go task. In our simultaneous discrimination task, the rats were confronted with the problem of competing responses to odor pairs containing odor A.

Generally, the overlapping task (A-C+) is expected to be more difficult to acquire than the nonoverlapping task (D+E-) because it interferes with the past learning experience (A+B-). The computational model has been used to demonstrate that the selective cholinergic suppression of intrinsic, but not afferent, fiber synaptic transmission during learning can prevent interference between different overlapping odor patterns. We therefore expected that the performance of the scopolamine rats would be impaired relative to their performance under the normal saline and methylscopolamine conditions. Loss of cholinergic modulation may impair odor pair acquisition due to the suppression of adaptation and depolarization of pyramidal cells and through effects on synaptic modification by the enhancement of long-term potentiation. We therefore presumed that there would be an interaction between drug and odor pair; that is, the hypothesized scopolamine impairment would be greater for the overlapping task (AC) than for the nonoverlapping task (DE).

## Method

### Subjects

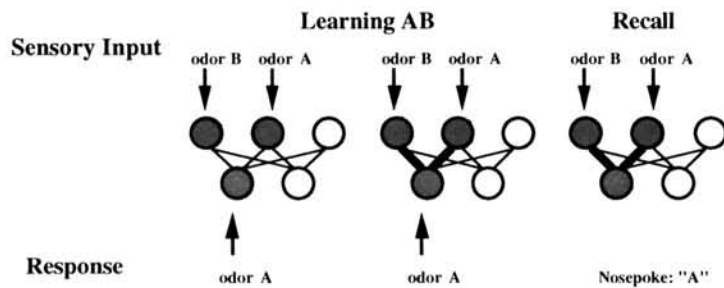
Adult male Sprague-Dawley rats ( $n = 19$ ; 250–350 g) were obtained from Charles River Laboratories (Wilmington, MA). They were individually housed and kept on a 12-hr light-dark cycle. The rats were allowed 1 week for acclimatization to the humidity- and temperature-controlled vivarium in the Psychology Department at Harvard University. To ensure motivation, they were water deprived for 48 hr prior to their initial training. After each session, the rats were given water ad libitum for 15 min to maintain their health, otherwise they were water deprived. Percentage of correct responses was the dependent measure and was calculated for each session, which consisted of 64 trials. All testing took place during the light phase of the cycle.

### General Paradigm

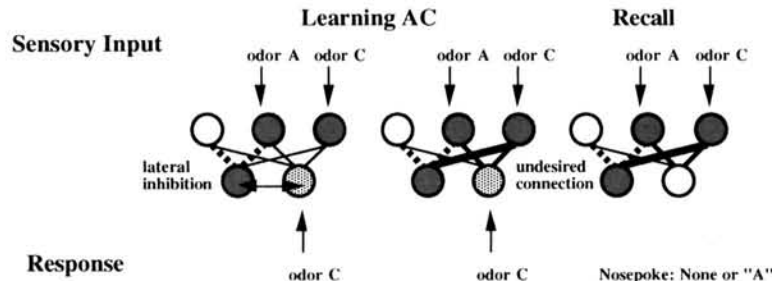
For each odor set, the rats were required to learn five odors (A, B, C, D, and E) that were paired into three different odor pairs (A+B-, A-C+, and D+E-). For each odor pair, two different odor cues were presented simultaneously from independent odor ports, and the port from which each odor was presented was counterbalanced. The rats learned three odor sets, for a total of 15 odors.

The first phase of the task entailed learning a baseline odor pair. The rats were required to learn odor pair A+B- (i.e., AB was reinforced if the rat responded to odor A). The experimental phase comprised two novel odor pairs: one that shared a component with the initial odor pair (overlapping) and one that did not share components (nonoverlapping). In the overlapping task, odor pair A-C+ was reinforced if the rat responded to odor C, and in the nonoverlapping task, D+E- was reinforced if the rat responded to

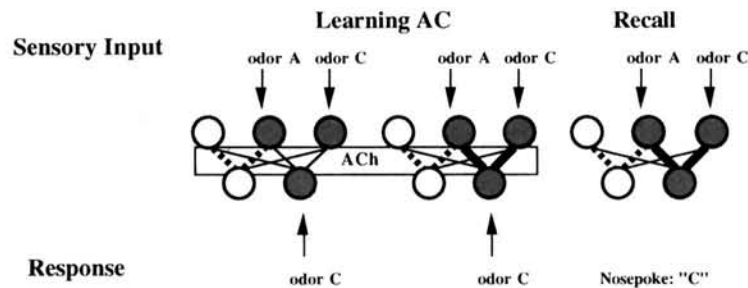
### (A) Learning initial odor pair



### (B) Overlapping odor pair (AC) without ACh – scopolamine group

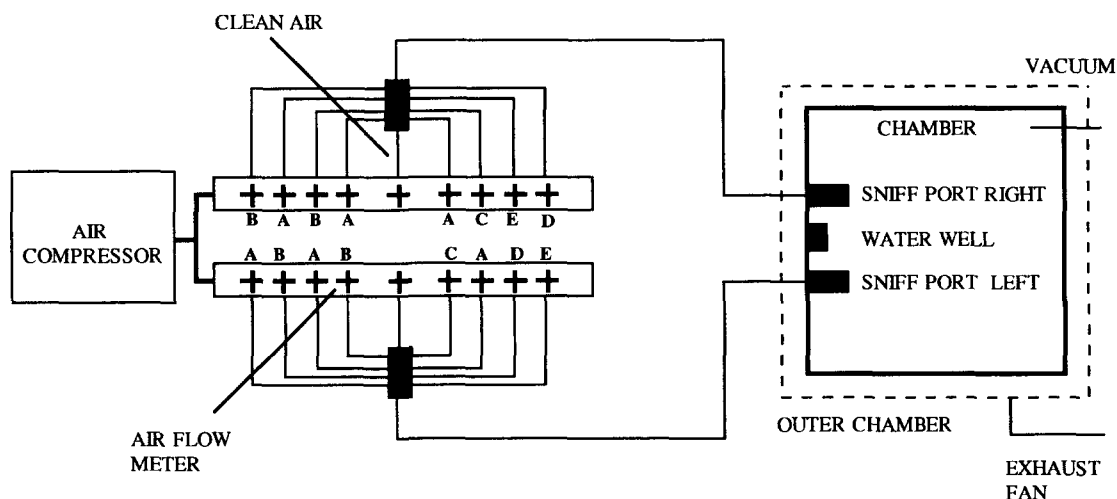


### (C) Overlapping odor pair (AC) with ACh – control groups



**Figure 1.** This simplified representation of a five-neuron olfactory network illustrates the basic elements of cholinergic modulation of associative memory for our proactive interference task. In the model, it is hypothesized that activity patterns in the olfactory cortex represent both the odor cue and the association with the behavioral response. This assumption is supported by *in vivo* recordings of pyramidal cells in the olfactory cortex during an olfactory discrimination task. Schoenbaum and Eichenbaum (1995) demonstrated neural activity for sensory and response activity in the same structure (olfactory cortex) during an olfactory discrimination task. In each group of neurons, the top row represents two neurons out of three being activated by sensory input (shading represents activation), and the bottom row shows neurons representing nose poke responses to odor ports containing the positive odor. During learning, a Hebbian learning rule strengthens the connections between the sensory units and the response unit (as shown by thicker connections). During recall, only the sensory input is present. The activity spreads along the previously strengthened connection, activating the other neuron originally activated by the learned input pattern. Thus, intrinsic connections, strengthened during learning, help complete the response to the initial learning experience. Panel A: During learning of the initial odor pair A+B-, odors A and B activate neurons in the top row, whereas the correct response to odor A activates the neuron in the bottom row. Strengthening of the connections between these neurons allows presentation of odors A and B to subsequently evoke the correct response (nose poke to A). Panel B: Learning the overlapping odor pair (A-C+) without cholinergic modulation (scopolamine). Encoding of this second odor pair (A-C+), which overlaps with the first odor pair (A+B-), suffers from interference due to recall of the first odor pair during learning. When odor pair AC is presented, the middle neuron immediately activates the neuron on the left via the connections strengthened by the first learning experience. This causes strengthening of undesired connections and may prevent strengthening of the desired connection due to lateral inhibition. Under these conditions, during recall the network results in no response or an "A" response instead of the "C" response. Panel C: Learning the overlapping odor pair (A-C+) with cholinergic suppression (normal saline and methylscopolamine). With acetylcholine effects present, suppression of intrinsic fiber synaptic transmission reduces interference between the overlapping components during acquisition. The suppression of all intrinsic connections in the network prevents the spread of activity across previously modified connections. The Hebbian learning rule can strengthen the connections between sensory neurons and the response neuron "C" without interference. Therefore, during recall, presentation of AC activates the "C" response.

## 16 CHANNEL FLOW-DILUTION OLFACTOMETER



*Figure 2.* Schematic diagram of the 16-channel, flow-dilution olfactometer that allowed simultaneous presentation of odor pairs for the associative memory task ( $A+B-$ ,  $A-C+$ ,  $D+E-$ ). The left section of the figure depicts the air compressor, two-way solenoid valves, and flow meters. Clean air flows continuously and odorized air flows when appropriate. On each trial, the left-right positions of two odors were selected according to a pseudorandom schedule and were counterbalanced within a session. The right section of the figure depicts the Plexiglas chamber surrounded by a sound-attenuating wooden enclosure with an exhaust fan to eliminate lingering odors. The chamber contained two conical odor ports and a water well made of chemically resistant Delrin material. There were infrared photobeams across the opening of both odor ports and the well.

odor D. The overlapping odor pair AC acted as the stimulus to produce proactive interference, and the nonoverlapping odor pair DE acted as the control for learning a novel odor pair while under the influence of a drug.

### Olfactometer

An olfactometer (odor generator and test chamber) constructed in our laboratory provided precise and fully automated control of the odor stimulus and the testing environment (Otto & Eichenbaum, 1992; Slotnick & Nigrosh, 1974). In our olfactometer, all procedural events were controlled and behavioral events recorded by a computer with custom-designed interfaces. Figure 2 depicts the 16-channel, flow-dilution olfactometer, in which the air flow system was controlled by flow meters and two-way solenoid valves allowing clean air to flow continuously at 2.5 liters per minute (LPM) and odorized air to flow at 2.0 LPM, when appropriate (Eichenbaum, Fagan, Mathews, & Cohen, 1988; Otto & Eichenbaum, 1992). On each trial, the left-right positions of two odors were selected according to a pseudorandom schedule and counterbalanced within a session. Odorized and clean air flowed through chemically resistant tubing.

An inner Plexiglas chamber ( $30.5 \times 30.5 \times 38$  cm) was surrounded by a sound-attenuating wooden enclosure with an exhaust fan for lingering odors. There was a vacuum line attached to the roof of the inner chamber to rid the chamber itself of odors. On the front panel of the chamber, there were two conical odor ports (2.5 cm diameter) made of chemically resistant Delrin material, placed 6 cm above the floor and 4 cm apart on either side

of the water well. The odor ports were placed 4 cm apart to promote independent presentation of each component of the odor pair. The water well (2.5 cm in diameter), which was also made of Delrin, was placed 12 cm above the floor. There was an infrared (IR) photobeam across the opening of both odor ports and the well. In front of these odor ports was a 1-cm Plexiglas barrier, which forced the rat to deliberately poke into the odor port rather than allowing it to accidentally break the IR beam across the odor port opening.

A 5-V, green LED positioned immediately over the well signaled reinforcement, and a 5-V, red LED positioned immediately under the water well and centered over the two odor ports signaled any incorrect response. The two LEDs were placed in spatially distinct places on the front panel because albino rats have poor vision. All of the behavioral responses were monitored by a computer.

### Odorants

The appendix lists all of the experimental odorants, all of which were provided by Sigma Chemical (St. Louis, MO) or The Body Shop (Wake Forest, NC).

### Operant Procedure

This was a discrete-trial operant task in which the rats were shaped to (a) move toward the back of the chamber while clean air was being presented, (b) approach odorized air at the front of the chamber, (c) place their nose in an odor port when odor was present, and (d) retrieve water by breaking the appropriate IR

photobeam after a nose poke to the reinforced odor. As the rats retrieved water from the well and as they moved away from the odor ports, clean air flowed through the system, and any odorized air left in the chamber was exhausted by vacuum. The experimenter controlled the trial onset, and the rats had to await the trial onset signal—odor present in the chamber.

During shaping, clean air and an odor were simultaneously presented from the two odor ports. The rats were initially shaped to nose poke the odor port from which the odor was being presented, versus no odor. If the rat correctly nose poked, then the green LED immediately over the well signaled the rat to break the water-well IR photobeam to receive 0.05 ml of water. If the rat made any incorrect response, then the red LED turned on for at least 4 s. A correct response consisted of the rat nose poking where the positive odor was present. An incorrect response consisted of one of the following behaviors: (a) a nose poke when no odor was present in the chamber (premature response); (b) a nose poke to the well; (c) a nose poke to the incorrect odor port (error of commission); or (d) no response within 10 s (error of omission).

Our task required the rats to respond directly to the discriminative stimuli with a nose poke to the odor stimulus. When the animal is required to respond directly to the discriminative stimuli, simultaneous discrimination tasks are easier than successive discrimination. An added benefit to simultaneous discrimination tasks is that they may also provide additional relational cues, which may help the animal to learn difficult discriminations (Mackintosh, 1974).

Eichenbaum (1992) noted that in a similar task, normal rats “consistently approached and sampled one sniffport first, then either performed a nose poke there, or approached and sampled the other sniffport” (p. 222). This observation guided the behavioral procedure designed for this specific task, in which the rats were not allowed to wait near the odor ports in the front of the chamber. Instead, they were forced to approach and sample the odors after pausing away from the ports, toward the back of the chamber. The next odor trial was not presented until the rats had moved away from the odor ports. The requirement of making the rat approach the odor from the back of the chamber and perform a simultaneous discrimination served to encourage the construction of a representation of the entire set of stimuli rather than independent odors.

Slotnick (1994) cautioned that even after near perfect performance had been achieved on the shaping trials, destructive behaviors reappeared when a more difficult discrimination task was introduced. In creating this simultaneous discrimination task, it was important to determine what design yielded optimal learning and allowed the rats to maintain a high level of performance once the task became more difficult. This is because learning under the influence of scopolamine was certain to make the task more difficult. A measure of response flexibility (e.g., trial was over and recorded as incorrect if the rats did not respond in 10 s) was included; this was thought to be necessary to separate the anticipated drug effects on memory from other known pharmacological effects, (e.g., speed of responding).

We anticipated behavioral and attentional impairments under the influence of the highest scopolamine dose (0.50 mg/kg) and consequently measured the proportion of errors of omission. After the highest dose, the operant procedure was slightly modified, so that there was neither a 10-s response criterion nor an incorrect response to the well. With this modified procedure, the next two sets of rats (0.25 and 0.125 mg/kg doses) were forced to nose poke an odor for every trial, and there was no longer a timing demand. Between trials, the rats were required to move away from the odor ports and await the onset of the next trial, signaled by an odor presentation.

### *Shaping Procedure*

The rats were first presented with simultaneous discriminations of odor versus no odor for the shaping procedure. They were required to nose poke the odor port where an odor was present and were presented with alternating blocks of 16 trials of left responses and 16 trials of right responses. As the rats attained a performance criterion of 18 consecutive correct responses in 20 trials, they were moved toward trials in which the side of odor presentation side was randomly alternated. During shaping, “correction” trials were presented to prevent the rats from developing a side bias. Training to randomly presented trials was continued until a performance criterion of 18 correct trials responses in a block of 20 trials was met. The dependent measure was percentage of correct responses.

Once all of the rats reached criterion on odor versus no odor, they then learned their first simultaneous odor discrimination task, in which two different odors were independently and simultaneously presented from both odor ports. Of the two odors, one was arbitrarily labeled the positive odor (A+B−). The rats indicated their choice with a nose poke to the odor port with the positive odor. Once the rats were trained to criterion, they learned an overlapping odor pair (A−C+) to criterion. Each session comprised 32 trials of odor pair AB and 32 trials of odor pair AC. The overlapping odor pair was presented during shaping to demonstrate that all rats were able to acquire the overlapping odor pair. It is important to note that once AC had been learned it existed in memory concurrently with AB, that is, learning of AC did not weaken the representation of the original odor pair in memory.

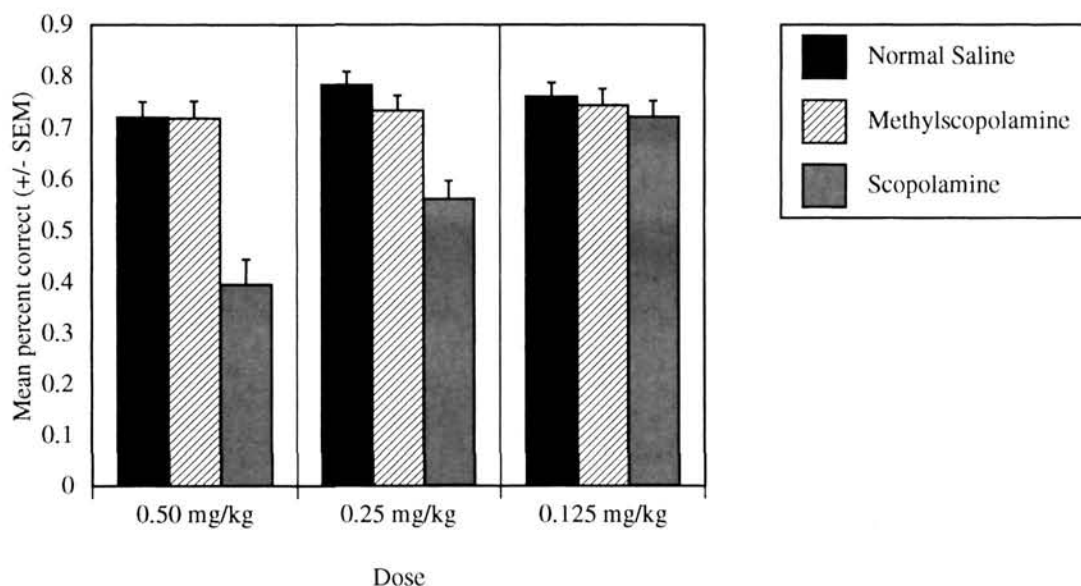
### *Experimental Procedure*

After shaping, the rats learned a new A+B− odor pair to criterion and were then randomly assigned to a drug condition, of which the experimenter was unaware. They were then tested on the two novel experimental odor pairs (overlapping and nonoverlapping) under the influence of the drugs. For four consecutive sessions, the rats were presented with 32 trials of A−C+ and 32 trials of D+E−, intermixed in a pseudorandom order. At the beginning of each of the experimental sessions, they were presented with 16 “reminder” A+B− trials. These reminder trials served only as an opportunity for the experimenter to observe any attentional impairments and adjust the trial onset accordingly. The dependent measure was percentage of correct responses.

### *Drug Administration*

The drugs scopolamine hydrobromide (scopolamine), a central muscarinic receptor antagonist, and N-scopolamine methylbromide (methylscopolamine), a peripheral muscarinic receptor antagonist, were dissolved in normal saline vehicle (0.9 g NaCl/100 ml H<sub>2</sub>O). Methylscopolamine does not pass through the blood-brain barrier and therefore served as a control for the effects of scopolamine in the peripheral nervous system. The drugs were administered 15 min prior to each test. For the experimental task, overlapping and nonoverlapping odor pairs, the rats were injected intraperitoneally with scopolamine, methylscopolamine or normal saline.

The first set of rats ( $n = 6$ ) received 0.50 mg/kg scopolamine, methylscopolamine, or normal saline. The next set of rats ( $n = 6$ ) received 0.25 mg/kg of each drug or normal saline, and the last set of rats ( $n = 7$ ) received 0.125 mg/kg of each drug or normal saline. Within each dose, each rat was administered each of the three drugs across three different odor sets. Across rats, drug dose order was



*Figure 3.* Mean percentage correct for each drug dose, collapsed across session and odor pair to demonstrate the Drug  $\times$  Dose interaction. As the scopolamine dose increased, the performance of the rats decreased. The scopolamine rats were significantly impaired while performing under the influence of the high- and midlevel doses (0.50 and 0.25 mg/kg). At the lowest dose (0.125 mg/kg), scopolamine rats' performance was comparable to their performance under the two control conditions.

controlled according to a latin square design, and each odor set was paired equally often with each drug condition. The only exception was in the 0.125 mg/kg dose, where one order was repeated due to the inclusion of an additional rat. If a rat did not reach criterion within the four consecutive drug-influenced sessions, it was trained to criterion on that odor set before advancing to the next drug group.

## Results

The percentage of correct responses for each experimental session, including all drug doses, was calculated and submitted to a  $4 \times 3 \times 2 \times 3 \times 3$  mixed analysis of variance (ANOVA) with three within-group factors (session, drug group, and odor pair) and two between-group factors (scopolamine order and dosage).

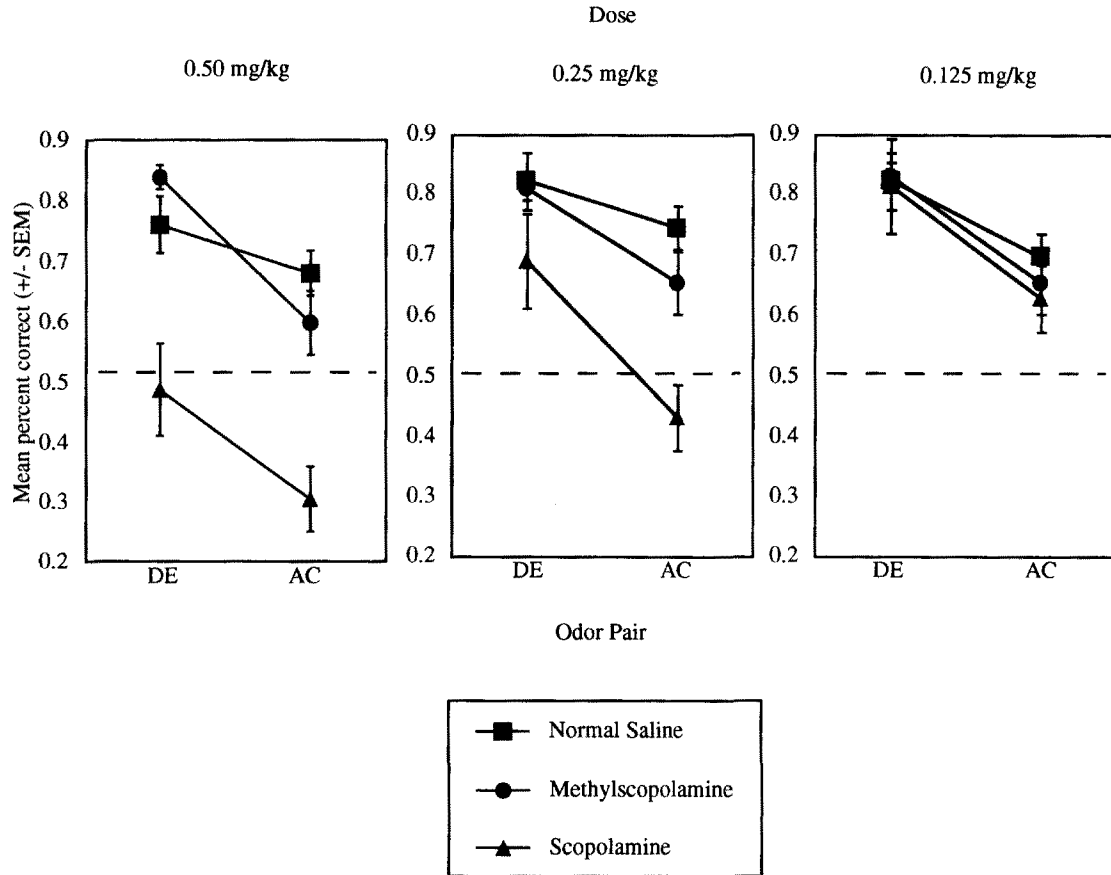
Scopolamine order was included in the analysis to determine whether the order in which the rats received scopolamine acted as a confound. Specifically, whether receiving scopolamine for the first odor set would have more impact on learning than receiving scopolamine on the second or third odor set. There was no main effect of scopolamine order,  $F(2, 14) = 0.244$ , and no significant interaction between scopolamine order and any of the other factors with which it was crossed. Whether the rats received scopolamine on their first, second, or third odor set did not affect the acquisition of the experimental odor pairs under the influence of the other drug conditions. Scopolamine order was thus collapsed and not considered as a factor for the following analysis.

The rats were presented with the experimental odors for four consecutive sessions. As predicted, there was a main

effect of drug group,  $F(2, 32) = 17.120$ ,  $p = .0001$  (normal saline:  $M = .756$ ,  $SD = .196$ ; methylscopolamine:  $M = .733$ ,  $SD = .224$ ; scopolamine:  $M = .569$ ,  $SD = .304$ ). These data demonstrate that the two control groups were comparable and the scopolamine rats were more impaired in acquiring the experimental odor pairs. There was no significant difference on performance between the two control groups, methylscopolamine and normal saline,  $t(32) = 0.634$ .

There was a main effect of dosage,  $F(2, 16) = 4.088$ ,  $p = .037$  (0.125 mg/kg:  $M = .743$ ,  $SD = .226$ ; 0.25 mg/kg:  $M = .693$ ,  $SD = .228$ ; 0.50 mg/kg:  $M = .612$ ,  $SD = .304$ ). As the drug dosage increased, the mean performance of all of the rats decreased. Figure 3, representing the Drug  $\times$  Dosage interaction, illustrates that as the scopolamine dose decreased, the performance of these rats increased until it was comparable to that of the two control groups,  $F(4, 32) = 3.820$ ,  $p = .012$ . There was also a main effect of odor pair,  $F(1, 16) = 54.438$ ,  $p = .0001$ . The overlapping odor pair A-C+, designed to undergo greater proactive interference from the initial odor pair A+B-, was more difficult to acquire than the nonoverlapping odor pair D+E- (odor pair DE:  $M = .768$ ,  $SD = .225$ ; odor pair AC:  $M = .604$ ,  $SD = .265$ ).

A focused contrast (Rosenthal & Rosnow, 1988, 1991) was used to decompose the predicted Drug  $\times$  Odor Pair interaction. We predicted that the scopolamine rats would be more impaired at acquiring the overlapping odor pair than the nonoverlapping odor pair relative to their performance under control conditions. The focused contrast yielded significant results,  $F(1, 32) = 54.401$ ,  $p = .0001$  (see Figure



*Figure 4.* Mean percentage correct for the overlapping and nonoverlapping odor pairs, collapsed across session, to demonstrate the Drug  $\times$  Odor Pair interaction. The two control groups for each odor pair show no significant difference between them for any of the doses. The scopolamine rats under the influence of the highest dose (0.50 mg/kg) were globally impaired relative to their normal saline performance. Under the influence of the midlevel dose (0.25 mg/kg), the scopolamine rats were selectively impaired on the overlapping task but not the nonoverlapping task, relative to their normal saline performance. With the lowest scopolamine dose (0.125 mg/kg), the rats' performance was comparable to their normal saline performance.

4). That is, scopolamine rats, specifically at the midlevel dose (0.25 mg/kg), were significantly impaired on the overlapping task but not the nonoverlapping task, relative to normal saline rats.

The highest dose of scopolamine (0.50 mg/kg) produced a global mnemonic deficit. These scopolamine rats were significantly impaired on acquiring odor pair DE ( $M = .488$ ,  $SD = .377$ ), perseverated on odor pair AC ( $M = .305$ ,  $SD = .267$ ), and continued to respond to odor A in the AC condition. Figure 4 depicts these rats' performance versus chance (50%) and shows that they performed significantly below chance on odor pair AC,  $t(23) = 3.580$ ,  $p = .0016$ , and at chance on odor pair DE,  $t(23) = 0.161$ . Rats in the normal saline condition performed significantly above chance for both odor pairs, AC:  $t(23) = 4.843$ ,  $p = .0001$ ; DE:  $t(23) = 5.618$ ,  $p = .0001$ .

The scopolamine rats were also impaired at the midlevel dose (0.25 mg/kg), but in this case they demonstrated a selective impairment. The scopolamine rats were signifi-

cantly impaired relative to the normal saline rats on acquiring odor pair AC,  $t(10) = 4.770$ ,  $p = .0008$ . In contrast, the scopolamine rats were not significantly different from their normal saline performance on odor pair DE,  $t(10) = 2.029$ ,  $p = .07$ . Figure 4 shows that the scopolamine rats performed at chance on odor pair AC,  $t(23) = 1.642$ ;  $M = .433$ ,  $SD = .200$ , and significantly above chance on odor pair DE,  $t(23) = 4.286$ ,  $p = .0003$ ;  $M = .691$ ,  $SD = .218$ . Rats in the normal saline condition performed significantly better than chance for both odor pairs, AC:  $t(23) = 6.055$ ,  $p = .0001$ ; DE:  $t(23) = 12.103$ ,  $p = .001$ .

The performance of rats administered the lowest scopolamine dose (0.125 mg/kg) was comparable to that of the normal saline rats, AC:  $t(12) = 1.786$ ; DE:  $t(12) = 0.232$ . Rats in both the scopolamine and normal saline conditions performed above chance on both odor pairs, scopolamine: AC,  $t(27) = 2.636$ ,  $p = .014$ ; DE,  $t(27) = 10.255$ ,  $p = .0001$ ; normal saline: AC,  $t(27) = 4.752$ ,  $p = .0001$ ; DE,  $t(27) = 10.241$ ,  $p = .0001$ . The pairwise comparisons of the

two control groups (normal saline and methylscopolamine) for each odor pair, within the predicted Drug  $\times$  Odor Pair interaction, show that there was no significant difference between them for any of the doses, 0.50 mg/kg: AC,  $t(10) = .631$ , DE,  $t(10) = .599$ ; 0.25 mg/kg: AC,  $t(10) = 1.345$ , DE,  $t(10) = .195$ ; 0.125 mg/kg: AC,  $t(12) = 1.110$ , DE,  $t(12) = .228$ .

Although the 0.50 mg/kg scopolamine rats were all able to reach criterion by the fourth session, further investigation was warranted because of their severely impaired performance. The response time on correct trials and an accuracy measure were calculated and submitted to two separate, repeated-measures ANOVAs.

The response time measurement on correct trials was submitted to a  $4 \times 3$  (Session  $\times$  Drug Group) repeated-measures ANOVA for the highest drug dose. There was no significant difference between the three drug groups on mean reaction time,  $F(2, 10) = 2.284$ . When the rats were under the influence of scopolamine, the response time for their correct nose pokes was comparable to when they were under the influence of either control drug.

The proportion of errors of omission [error of omission / (error of commission + error of omission)] was calculated for the highest drug dose. This accuracy measure was submitted to a  $4 \times 3$  (Session  $\times$  Drug Group) repeated-measures ANOVA. There was no significant difference between the three drug groups on the proportion of errors of omission committed by each drug group collapsed across odor pair,  $F(2, 10) = 3.000$ . In each trial, these rats were just as likely to respond under the influence of scopolamine or either control drug.

## Discussion

Scopolamine did produce an overall impairment relative to the performance of the rats in the two control conditions—normal saline and methylscopolamine. Rats in the two control conditions performed comparably under all doses of methylscopolamine. In addition, our task was able to induce proactive interference: there was a main effect of odor pair. The rats found the overlapping odor pair (A–C+) more difficult to acquire than the nonoverlapping odor pair (D+E–). The highest scopolamine dose (0.50 mg/kg) created a global mnemonic deficit, so that these rats could only perseverate while acquiring the overlapping odor pair and perform at chance while acquiring the nonoverlapping odor pair. The midlevel scopolamine dose (0.25 mg/kg) created a selective impairment, that is, there was a nonsignificant relationship between the normal saline data and the scopolamine data on the acquisition of the nonoverlapping odor pair, whereas acquisition of the overlapping odor pair for the scopolamine rats was significantly impaired relative to saline. No significant difference was found between any of the drug groups for either odor pair at the lowest scopolamine dose (0.125 mg/kg).

Electrophysiological studies in the olfactory cortex have demonstrated that ACh selectively suppresses the synaptic transmission of intrinsic fibers relative to afferent fibers

(Hasselmo & Bower, 1992). As described in the introduction and in Figure 1, this suppression might prevent interference from previous associations during storage of a new association. Blockade of this suppression could underlie the selectivity of the scopolamine impairment for the acquisition of the overlapping odor pair versus the nonoverlapping odor pair under the influence of the midlevel dose (0.25 mg/kg). In addition, ACh has been shown to enhance activity-dependent synaptic modification (Hasselmo & Barkai, 1995), which may result from suppression of the normal adaptation of firing by pyramidal cells to a sustained depolarizing current injection (Barkai & Hasselmo, 1994), which would enhance both pre- and postsynaptic activity, thereby increasing LTP. Scopolamine blockade of the ACh enhancement of synaptic modification could underlie the generalized scopolamine impairment of learning at the high dose (0.50 mg/kg).

Presently, there is no commonly accepted psychological or physiological mechanism to explain the antimuscarinic-induced mnemonic deficits. It has been suggested that antimuscarinic drugs reduce stimulus discriminability or alter the sensory processing of conditioned stimuli. Therefore the question still exists in the literature: Do anticholinergic drugs affect mnemonic strategies of the animal or do they allow for inadequate stimulus discriminability and attention to the stimuli (Hagan & Morris, 1989; Inglis & Fibiger, 1995)? Scopolamine does not affect recall of material learned prior to the administration of the drug, only acquisition of new material, which argues against the rats' inability to properly attend to and discriminate stimuli (Hunter & Murray, 1989).

Under the influence of the highest dose of scopolamine (0.50 mg/kg), the rats were able to reach the strict response requirement of eighteen consecutive correct responses in twenty trials. Therefore, one could assume that these rats were able to appropriately attend to and discriminate the odor stimuli. We also demonstrated that, under the influence of the highest drug dose, the scopolamine group did not differ from the control groups on response time or proportion of errors of omission committed on correct trials.

Orsetti, Casamenti, and Pepeu (1996) demonstrated that activation of the forebrain cholinergic pathways (indicated by an increase in hippocampal and parietal cortex extracellular ACh levels) occurred during the acquisition of a rewarded operant response in naive rats but did not accompany the performance of the same response in trained rats. This may serve as an indication of an intense activation of the cholinergic forebrain projections related to the encoding of new stimuli. This finding supports our assumption that presentation of the novel odor pair triggers suppression of the intrinsic connections. Visual, auditory, olfactory, and tactile stimuli elicit increases in either cortical or hippocampal extracellular ACh levels, increases that differ in magnitude according to the type of stimulation (Inglis & Fibiger, 1995). Specifically, novel and conditioned visual and auditory stimuli can produce parallel increases in frontal cortical and hippocampal ACh release. Also, the increases in ACh produced by unconditioned stimuli are significantly reduced by habituation (Acquas, Wilson, & Fibiger, 1996). These

studies indicate the important role of the cholinergic system for the initial stages of learning and that these sensory stimulation-induced increases in cortical and hippocampal ACh release are not an inherent property of the stimulus presented. Presumably, scopolamine, by enhancing the strength of intrinsic transmission, could increase the interference between overlapping patterns during retrieval as well, although the effects would be more severe on acquisition because of accumulation across trials. Sohal and Hasselmo (1998) demonstrated that a change in the strength of intrinsic transmission can assist in disambiguation of stored sequences during retrieval.

Our behavioral results support the prediction that the physiological effects of ACh can reduce interference between stored memories during learning. Although the influence of systemic administration of an anticholinergic drug does not inform us in which brain regions ACh affects this particular task, we assume that the olfactory cortex is an important locus for ACh effects on olfactory learning. In support of our assumption, Schoenbaum and Eichenbaum (1995) demonstrated the firing properties of single pyramidal neurons in the olfactory cortex in behaving rats performing on an olfactory discrimination task. They demonstrated olfactory pyramidal cell firing for both odor sampling and water poke cells, indicating that olfactory cortical neurons encode the physical characteristics of the odor during sampling and maintain a representation of it during the response. Present studies in our laboratory are examining the specific role of the olfactory cortex in our simultaneous discrimination task.

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## Appendix

### List of Odorants From Which the Experimental Odors Were Chosen

Almond oil	n-Butyl glycidyl ether
Citronellal	Geranium oil*
Methyl salicylate	Trimethyl acetic anhydride
Aminoacetophenone	Caproic acid ethyl ester
Clove oil	Honeysuckle oil*
Phenylacetaldehyde	Valeric acid ethyl ester
Amyl acetate	Cineole
Rose oil*	Levulinic acid ethyl ester
Bay oil	Vanilla oil*
Gardenia oil*	Cinnamon oil
Rosemary oil*	Limonene
Benzylaldehyde	Citral
Geraniol	Linalool
Strawberry oil*	Ethylene glycol monoethyl ether

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\* Provided by The Body Shop

Received March 17, 1999  
 Revision received June 29, 1999  
 Accepted July 22, 1999 ■