

EFFECTS of scopolamine on performance of a delayed matching-to-sample task and on the properties of neurons in anterior-ventral inferior temporal (IT) cortex were examined in two monkeys. Both monkeys were impaired on the task after systemic administration of scopolamine, suggesting that scopolamine disrupts recency memory. Despite the behavioral deficit, neurons in IT cortex, a region having an important role in visual memory and neuronal properties consistent with that role, were largely unaffected by scopolamine. This dissociation between the behavioral and neuronal effects of scopolamine indicates that the drug either acts at a different site or disrupts unobserved mechanisms at the IT site.

Key words: Scopolamine; Memory; Macaque monkey; Inferior temporal cortex

Scopolamine affects short-term memory but not inferior temporal neurons

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Introduction

There is an abundance of research on the behavioral effects of scopolamine, a muscarinic receptor blocker. Systemic injections of scopolamine impairs performance on memory tasks in a variety of species (see review by Fibiger¹). Based on these results, it has been proposed that the cholinergic muscarinic system plays a central role in learning and memory. However, the site of scopolamine's action on such processes is not known.

In monkeys, inferior temporal (IT) cortex is important for normal memory of visual stimuli. Monkeys with damage to IT cortex, particularly the anterior-ventral portion, have difficulty in retaining visual information.^{2–4} Furthermore, IT neurons have properties consistent with a role in storage of visual memories.^{5–11} Their responses are determined jointly by the current visual stimulus and stored memory traces. Typically, the responses of IT neurons are suppressed when a current stimulus matches one held in memory even when multiple stimuli intervene in the retention interval.^{8,11} It has been proposed that the cholinergic system plays an important role in memory formation in IT cortex.¹²

Monkeys with damage to IT cortex are impaired in performance of two variants of delayed matching-to-sample (DMS) tasks, one of which is dependent on recency memory and the other on recognition memory. In DMS tasks, presentation of a sample stimulus is followed (after a delay) by two test stimuli, and the monkey chooses the stimulus that matches (or does not match) the sample. The recency version uses a small set of familiar stimuli that are used repeatedly, requiring the monkey to judge which stimulus was seen most recently. The recognition version uses novel stimuli on each trial, allowing the animal to base its decision on relative novelty and familiarity. Following scopolamine injection, monkeys exhibit deficits in recognition memory.¹³ In this study we assess scopolamine

effects on performance of a recency memory task as well as on the properties of IT neurons during performance of a recency memory task. This task was identical to one we used previously to explore the role of IT neurons in short-term memory.^{8,11}

Materials and Methods

All experiments were approved by the NIMH Institutional Animal Care and Use Committee. Two rhesus monkeys weighing 8 kg and 5 kg were used. A recording chamber was implanted above anterior-ventral IT cortex using coordinates derived from Magnetic Resonance Imaging. The localization of recording sites in anterior-ventral IT cortex was further confirmed histologically in one monkey.

Each animal alternated between a drug session on one day and a placebo session the next day. For a drug session, the animal received an IM injection of neostigmine methylsulfate ($10 \mu\text{g kg}^{-1}$) followed, after 5 min, by an IM injection of scopolamine ($10 \mu\text{g kg}^{-1}$). The neostigmine was to control for peripheral effects of the scopolamine. For a placebo session, the animals received two injections of equivalent volumes of saline. Behavioral and neuronal data collection began 10 min after the second injection and typically continued for 1.5 to 2 h. Action potentials of one or two single units, discriminated from a multi-unit signal by a spike sorter, were recorded in each session.

The task was a modified version of a delayed matching-to-sample (DMS) task. On each trial, while the monkey grasped a bar and fixated a small (0.2 deg) spot, a sample stimulus was presented at the center of gaze followed by sequential presentation of one to five test stimuli. When one of the test stimuli matched the sample, the monkey released the bar to receive an orange juice reward. Each stimulus was presented for 500 ms, with a 700 ms delay between the offset of one stimulus and onset of the next. The monkey was

required to maintain fixation of the fixation target throughout the trial. Within a trial, the only stimulus that was repeated was the stimulus that was used as the sample and the match; all the nonmatching stimuli were different from one another. The stimuli were a set of six complex, multi-colored pictures 1–3 deg in size chosen from a pool of 228 stimuli. The same stimulus appeared as a match on some trials and as a nonmatch on other trials. Once a stimulus was used in a session, it was not used again. An error was defined to be either release of the bar to a nonmatching stimulus or failure to release to the matching stimulus. Neuronal data were analyzed largely from trials in which the animals made a correct behavioral response, although neuronal responses on some error trials were examined to determine whether they differed from those on correct trials.

Neuronal responses to matching and nonmatching stimuli were evaluated with a *t*-test. However, a significant difference between these responses does not, in itself, provide a measure of how useful the difference would be for performance of the task. To help answer this, we employed discriminant analysis, which gave a measure of how accurately a stimulus could be classified as a match or a nonmatch based on the neuron's response on each trial. The analysis estimates the underlying distributions of responses and classifies a given response as signifying a match or nonmatch according to the probability that it was a member of one or the other distribution. The more the distributions overlap, the poorer the classification performance and therefore the less information. To test the possibility that scopolamine disrupted a "temporal code" conveying information about the match-nonmatch status of a stimulus,¹⁰ we repeated the discriminant analysis using the first three principal components (PCs) of the spike trains as a multivariate response measure.

When statistical classification procedures are applied to the same data to which the parameters of the classification algorithm fit, the classification scores will be biased. To correct for this bias, we used the cross-validation method. The discriminant functions were estimated on half the data (randomly chosen) and then the functions were fixed and applied to the other half of the data.

Results

Neuronal and behavioral data were collected over 20 experimental sessions with scopolamine administration and 18 control sessions with saline administration. These included 10 scopolamine sessions for each animal as well as 11 saline sessions for monkey A and 7 saline sessions for monkey B. Performance on the task was impaired under scopolamine. Across monkeys, the average performance in the scopolamine sessions was 67.1% correct, which was significantly

worse than the average performance of 87.4% correct during the placebo sessions ($t = 5.80, p < 0.001$). The majority of errors in drug and placebo sessions were incorrect bar releases, that is, incorrectly identifying a nonmatch stimulus as a match. A two-way ANOVA on the daily performance data with animal (A or B) and drug (scopolamine or saline) as factors revealed a significant effect of drug ($F = 6.136, p < 0.001$) but no significant effect of animal ($F = 0.97, p = 0.923$) and no significant interaction between these factors ($F = 0.109, p = 0.914$). Thus, both monkeys were similarly impaired by scopolamine.

Figure 1 shows the effects of intervening stimuli on performance. The disruption of performance by scopolamine was not exacerbated by increasing the number of stimuli (and delay) that interposed between the sample and the match. A two-way ANOVA was computed separately for each monkey, with scopolamine or saline (drug) and number of intervening stimuli (position) as factors. This analysis revealed a significant effect of drug for each animal (monkey A: $F = 3.54, p < 0.0001$, monkey B: $F = 3.1, p = 0.003$), and a significant effect of position for each (A: $F = 5.47, p < 0.001$; B: $F = 5.41, p < 0.001$), but no interaction between these factors (A: $F = 0.611, p = 0.542$; B: $F = 0.547, p = 0.586$).

Scopolamine also interfered with the animals' ability to maintain fixation. The mean number of trials in which the animals broke fixation was 39.5% and 14.5% during drug and placebo sessions respectively, a highly significant difference ($t = 4.64, p < 0.0001$). These errors were typically sudden, large amplitude saccades away from the fixation point rather than a chronic difficulty in maintaining eye position. As already indicated such trials were excluded from the

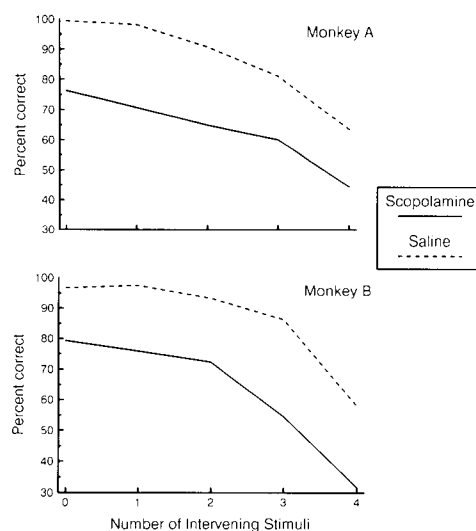


FIG. 1. Performance of monkeys under scopolamine and saline conditions as a function of number of stimuli that intervened between the sample and the match.

behavioral analysis. A total of 57 visually-responsive cells were recorded from the two monkeys during performance of the DMS task. Data were collected from 31 cells and 26 cells during scopolamine and placebo sessions, respectively.

To determine whether a neuron responded to a stimulus, we compared the firing rate to the stimulus when it appeared as a sample with the neuron's baseline firing rate. A similar number of stimuli were effective at eliciting a visual response during scopolamine and saline sessions: 42% (78/186) and 52% (81/156) under scopolamine and saline, respectively. The difference in proportion of effective stimuli between the groups fell short of significance (chi-square test, $p = 0.06$). We have previously found that IT neuronal responses to stimuli that match an item held in memory are suppressed relative to the responses to nonmatching stimuli.^{8,11} We found the same effects in the present study for cells recorded under drug and placebo conditions. According to a t -test, the suppression was significant for 22% (17/78) of the effective stimuli under scopolamine conditions and 15% (12/81) under placebo conditions. The difference in proportion of stimuli that exhibited a significant match-nonmatch effect in the two conditions was not significant (chi-square test, $p = 0.25$). The average responses of the population of IT neurons to stimuli that exhibited a significant match-nonmatch effect are shown in Figure 2 for both scopolamine and placebo sessions. For both, responses to matching stimuli were suppressed compared with nonmatching stimuli even when up to three stimuli intervened between the stimulus and the sample (drug: $t = 2.90$, $p = 0.010$; placebo: $t = 3.44$, $p = 0.0056$). These results are consistent with our previous study in untreated monkeys (Miller *et al*, 1991; Miller *et al*, 1992). Further-

more, an analysis of the responses to the stimuli that elicited a significant match-nonmatch effect during drug sessions showed that there was no difference between responses on correct and incorrect trials. There were not enough incorrect trials during the placebo sessions to conduct this analysis. Thus, under both scopolamine and placebo conditions, there was mnemonic modulation of the responses of IT neurons, even on incorrect trials.

As a measure of the amount of information about the sample that was carried in the responses to the test stimuli, a discriminant analysis was computed separately for each cell and each stimulus. First, the match-nonmatch classification scores for responses to all effective stimuli were compared, regardless of whether or not these stimuli had elicited a significant effect. The mean classification performance under the scopolamine and placebo conditions was 53.0% and 52.2% correct (chance = 50%), respectively. This was not a significant difference (t -test, $t = 0.776$, $p = 0.44$). We next compared the classification scores of the responses for just the stimuli with significant effects. The mean classification performance for the significant stimuli under scopolamine and saline conditions was 58.36% and 60.03%, respectively, which was not a significant difference ($t = 0.94$, $p = 0.356$). We found virtually identical results using the first three PCs of the spike trains in the discriminant analysis. Thus, IT neurons conveyed similar information about whether a stimulus was a match or nonmatch in the drug and placebo sessions.

The sensory properties of the neurons were also unaffected by scopolamine. We computed the discriminant analysis on the neuronal responses to sample stimuli to determine how much sensory information about the stimulus was carried in the responses. First, the classification scores for all neurons were compared. The mean classification performance under the scopolamine and placebo sessions was 23.7% and 22.7% correct, respectively (chance = 16.7%), which was not a significant difference ($t = 1.0$, $p = 0.328$). Next, we compared the classification scores for just those neurons that showed significantly different responses with the six stimuli based on the discriminant analysis. The test was significant for 42% (13/31) of the neurons recorded under scopolamine and 50% (13/26) of those recorded under saline conditions. The proportions were not significantly different (chi-square test, $p = 0.54$). The mean classification performance for the scopolamine and saline groups was 29% and 28.17% correct, respectively, which was not a significant difference ($t = 0.37$, $p = 0.71$). Thus, scopolamine did not attenuate the sensory information carried in the responses of IT neurons.

We did find one apparent neuronal effect of the drug. The neurons recorded under scopolamine were more responsive than neurons recorded under placebo conditions. The average response to effective sample stim-

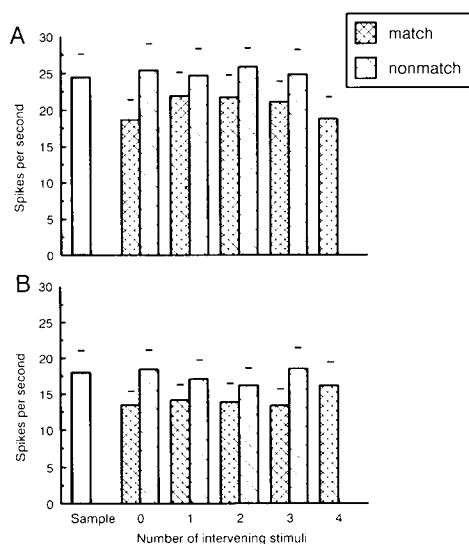


FIG. 2. Average responses across cells to the same set of stimuli appearing as samples and as matches and nonmatches following different numbers of intervening stimuli. The small horizontal line above each bar shows the standard error of the mean.

uli in the scopolamine condition was 21.3 spikes⁻¹, which was significantly greater than the average response of 13.8 spikes s⁻¹ in the placebo condition ($t = 3.03$, $p = 0.003$). By contrast, there was no significant difference between the spontaneous activity during scopolamine (12.6 spike s⁻¹) and placebo (10.2 spikes s⁻¹, $t = 1.02$, $p = 0.3$).

Discussion

We found that systemic administration of scopolamine interfered with performance of a recency memory version of a DMS task. The impairment was not aggravated by increasing the number of stimuli and, consequently, the time that intervened between the sample and the matching stimulus. Despite the behavioral impairment, the mnemonic and sensory information communicated by IT neurons was unaffected by scopolamine. Under scopolamine, neurons appeared to have a larger visual response, but this apparent change in responsiveness did not effect the mnemonic and sensory information conveyed by the neurons. The properties of IT neurons recorded from both conditions were similar to those found in our previous study.^{8,11}

The importance of IT cortex in visual memory is well established. In the present study, however, we found a dissociation between the influence of scopolamine on behavior and on IT neuronal properties. There are at least two possible explanations for this dissociation. First, the site of action of scopolamine may not be IT cortex. Previously, we proposed that the differential responses of IT neurons to matching and nonmatching stimuli are interpreted by neurons in a "decision network" (downstream from IT cortex) that identifies the matches.¹¹ It is possible that this hypothetical decision network is the site of action. In fact, we found no difference in IT neurons' responses on correct and incorrect trials, suggesting that the "error" occurs at a location downstream from IT cortex.

The second possibility is that scopolamine interferes with task performance by disrupting a memory mechanism in IT cortex that was not detected in our experiment or by disrupting mechanisms other than those underlying memory. Memory is not a unitary phenomenon, but rather is based on a collection of different, often independent, neural systems.^{12,14} Differences in tasks and training history may lead to different mnemonic mechanisms supporting task performance. Thus, it is possible that performance of this memory

task was not critically dependent on the observed modulations of IT neuron responses but rather on an unobserved IT mechanism. Furthermore, in addition to memory, scopolamine seems to affect a wide variety of behavioral processes, including attention, perception and movement. In fact, we found that in addition to producing a difficulty in identifying the match stimulus, scopolamine also interfered with the animals' ability to maintain fixation. The lack of an effect on IT neurons' sensory properties suggests that perceptual and attentional mechanisms related to form vision are unaffected. Which mechanisms are affected by scopolamine seems to depend largely on the task employed. Indeed, Fibiger argued that muscarinic receptor blockade must affect a variety of neural mechanisms since muscarinic receptors are widely distributed; whether specific mechanisms are affected may depend on requirements for local muscarinic actions which will depend, in turn, on environmental or task demands.

Conclusions

The results demonstrate that scopolamine affects performance of a recency memory task yet does not affect mnemonic and sensory information conveyed by IT neurons. These results suggest that scopolamine acts at a different site or affects unobserved mechanisms at the IT site.

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