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# Evidence for a Direct Cholinergic Involvement in the Scopolamine-Induced Amnesia in Monkeys: Effects of Concurrent Administration of Physostigmine and Methylphenidate with Scopolamine

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BARTUS, R. T. *Evidence for a direct cholinergic involvement in the scopolamine-induced amnesia in monkeys: Effects of concurrent administration of physostigmine and methylphenidate with scopolamine.* PHARMAC. BIOCHEM. BEHAV. 9(6)833–836, 1978.—Three male, test-sophisticated rhesus monkeys were run under various drug conditions to evaluate the amnesia induced by low doses of the anticholinergic scopolamine. The anticholinesterase physostigmine partially, but reliably reversed the amnesia in all three monkeys, while the catecholaminergic stimulant methylphenidate potentiated the behavioral deficits produced by scopolamine. These results provide further support for the idea that the amnesia produced by scopolamine is due to a direct dysfunction in central cholinergic mechanisms, and is not due to some general effect involving arousal, attention, etc. These results were then discussed as they may relate to the amnesia which occurs naturally in aged humans, and nonhuman primates.

Memory Short-term memory Cholinergic mechanisms Neurochemical mechanisms of memory
Scopolamine Primate behavior Physostigmine Drug interactions Methylphenidate Anticholinergics

RECENT research with human subjects [9, 11, 13, 14, 15] and non-human primates [2,6] has demonstrated that low doses of the anti-cholinergic scopolamine induce an amnesic state qualitatively similar to that which occurs naturally in aged humans [10,11] and monkeys [1, 2, 5]. Similar blockade of the dopaminergic system, via haloperidol, does not produce this selective effect on monkey memory [3,4]. The non-human primate studies enjoy the unusual benefit of having been collected in the same apparatus and under very similar experimental conditions, thus making cross-study comparisons more meaningful and less problematic. Taken collectively, they suggest that cholinergic mechanisms are critically involved in the normal expression of recent, or short-term memory, and that some specific dysfunction in these same mechanisms may play a primary role in the cognitive impairments that occur with old age [2,3].

However, an alternative explanation to the scopolamine deficit has been raised. Rather than impairing performance by disrupting cholinergic processes involved in memory, per se, it could be that scopolamine disrupts performance because of its more general effects on arousal, attention, and/or motivation. Bartus and Johnson [8] addressed this issue and argued that the highly specific nature of the deficit they reported (limited to those conditions actually requiring memory) made such a possibility unlikely. More recently, Drachman [9,11] empirically evaluated this idea using human subjects. He found that while the anti-cholinesterase physostigmine was effective in substantially reducing the scopolamine-induced amnesia, the central nervous system stimulant amphetamine was not. Drachman, therefore, concluded that simply increasing general levels of arousal or attention (presumably via catecholaminergic mechanisms) is not sufficient to reverse the amnesia induced by scopolamine in humans. Ghoneim and Mewaldt [14] also recently reported a significant reduction of scopolamine-induced amnesia with concurrent administration of physostigmine in humans. These studies, therefore, suggest that the amnesia induced by scopolamine is due to a direct interference with necessary cholinergic mechanisms. Yet, because this issue bears directly on an eventual understanding of the

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neurochemical basis of memory, and the relationship between these processes and the declines observed naturally with aged subjects, additional confirmation of this notion would seem to be important and worthwhile. The following study therefore attempted to confirm and extend the human clinical findings by evaluating the nature of the scopolamine-induced amnesia in rhesus monkeys. More specifically, the anticholinesterase physostigmine and the catecholaminergic stimulant methylphenidate were assessed for their effects on the scopolamine-induced amnesia. All testing was performed under tightly controlled laboratory conditions, using each subject as its own control, in the same apparatus and procedure previously used to study non-human primate memory under conditions of cholinergic blockade and advanced age [2, 3, 4, 6, 7].

## METHOD

### *Animals*

Three male, feral-born rhesus monkeys were used for this research. All monkeys appeared to be healthy and were estimated to be between the ages of 4 and 7 years old. All had participated in psychopharmacological studies for a minimum of two years prior to this study.

The monkeys were housed individually in animal colony facilities fully accredited by the American Association for Animal Laboratory Care. Their diet of Purina monkey chow and fresh fruit was given to them once per day immediately following completion of their behavioral testing.

### *Apparatus and Procedure*

The apparatus used in this research was the Automated General Experimental Device (AGED), illustrated in Fig. 1. Because it has been described in several recent reports [2, 6, 7] a detailed description will not be provided here.

A food-reinforced, delayed response procedure was used, similar to that used previously in related studies [2, 3, 4, 5, 6, 7]. Seventy-five trials were run per session, 5 days per week. As in all previous studies using this apparatus, each trial was subject-initiated and paced, beginning as soon as the monkeys placed their face into the observation window (Fig. 1).

Delay intervals of 0, 30 and 60 sec (separating off-set of stimulus cue and opportunity to respond) were quasi-randomly distributed throughout each test session.

### *Dosing Procedure*

Four different drug conditions, in addition to several non-drug control sessions, were administered to each monkey. The four drug conditions consisted of: (a) scopolamine, (b) scopolamine given simultaneously with methylphenidate, (c) scopolamine given simultaneously with physostigmine, and (d) methylphenidate alone. To help simplify the experimental design, the doses of each drug were selected for each monkey on the basis of individually titrated, pilot tests. The dose of scopolamine (0.015 to 0.02 mg/kg) was selected to optimize the memory-specific effects known to exist, while minimizing the more general, non-memonic effects known to occur with higher doses [6]. The dose of methylphenidate (0.0125 mg/kg) was selected on the basis of previous work in our laboratory showing that this dose was well below those known to impair performance on delayed response tasks [5], but yet not so low that beneficial effects might be impossible to demonstrate. Finally, the dose of physostigmine (0.02 to 0.03 mg/kg) was selected on the basis of previous research, defining the highest dose just *below* where impairments would begin to occur [3].

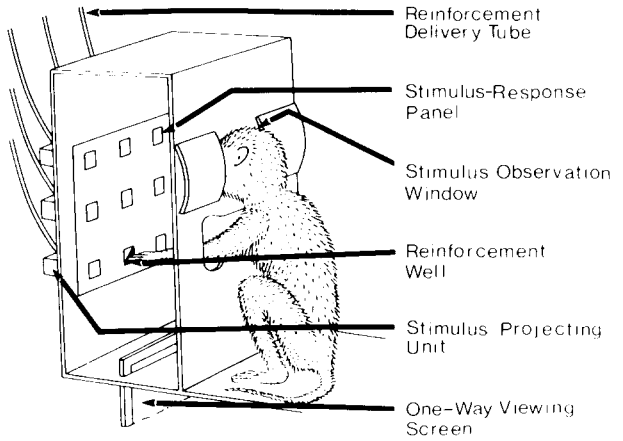


FIG. 1. Artist's conception of monkey working in the Automated General Experimental Device (AGED) used in this research. The important features of the apparatus are labeled, including a stimulus observation window (through which the monkeys look to initiate each trial), a 3×3 matrix of stimulus/response panels (one of which is illuminated each trial, and to which the monkey must respond to obtain a food reinforcement), and a one-way viewing screen (which serves to visually and/or physically isolate the monkey from the stimulus/response matrix, depending upon its position and the amount of backlighting).

All injections were given via the intramuscular route, 30 min prior to the initiation of the behavioral test. The order that the four drug conditions were given to each monkey was randomly determined to control for possible order effects. No more than two drug sessions were administered per week, with a minimum of 3 days separating each drug session. All non-drug sessions were pooled to improve the estimate of non-drug control performance for each monkey.

## RESULTS

The results of this study reflect excellent consistency across animals, in that all 3 monkeys displayed the same relative effects of the 5 experimental treatments, illustrated in Fig. 2. The most apparent and predictable finding was that low doses of scopolamine produced a selective amnestic effect on performance. That is, little or no impairment on response accuracy occurred on the 0 sec condition, where little or no short-term memory was required, but, as the stimulus information had to be held in memory for longer periods of time (i.e., 30 and 60 sec), progressively greater impairments occurred. These results thus replicate previously reported findings under similar experimental conditions [6]. The effects of combining physostigmine with scopolamine reduced the amnestic effects of scopolamine in all 3 monkeys, by an overall mean of over 50% on the two memory-dependent intervals (i.e., 30 and 60 sec). On the other hand, combining the central nervous stimulant, methylphenidate, with the same dose of scopolamine, potentiated the amnestic effect in all 3 cases, at a dose where methylphenidate alone produced no measurable behavioral effects.

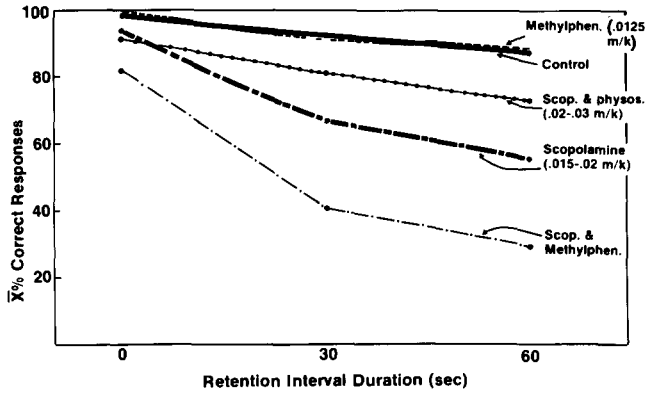


FIG. 2. Effects of scopolamine vs. non-drug control (two darker lines) on primate short-term memory. Note progressively greater debilitating effects of scopolamine as the duration of the retention interval is increased (i.e., the time between when the stimulus light is extinguished and when the monkey can execute a choice response). Concurrent administration of physostigmine partially reversed the scopolamine-induced amnesia, while concurrent administration of methylphenidate potentiated the deficit, at a dose of methylphenidate which produced no measurable effects when given alone.

These general impressions were confirmed by a 2-way analysis of variance (drug treatment and retention interval), with repeated measures (animals) on both factors [21]. Although the drug×retention interval interaction did not quite satisfy conventional levels of statistical reliability, $F(8,16)=2.49$, $p<0.10$, a highly reliable overall effect of drug treatment was observed, $F(4,8)=20.37$, $p<0.001$, as was an effect of retention interval duration, $F(2,4)=67.73$, $p<0.001$. Individual Newman-Kuels tests for differences among treatment means confirmed the fact that scopolamine indeed impaired performance when compared to non-drug control scores (critical difference needed=15.77, critical difference obtained=20.81, $df=8, 16$, $p<0.05$). Simultaneous administration of physostigmine and scopolamine was not reliably different from control scores, however (critical difference needed=12.72, critical difference obtained=10.20, $df=2, 8$, $p>0.05$), demonstrating a marked reduction of the scopolamine-induced amnesia. On the other hand, methylphenidate reliably potentiated the scopolamine impairment, for this drug combination was significantly inferior to scopolamine alone (critical difference needed=12.72, critical difference obtained=21.17, $df=2, 8$, $p<0.05$), while the same dose of methylphenidate, when given alone, was no different from control (critical difference needed=12.72, critical difference obtained=0.24, $df=2, 8$, $p>0.05$).

## DISCUSSION

These results demonstrate that the amnesia produced in rhesus monkeys by low doses of the anticholinergic scopolamine can be partially reversed by the anticholinesterase physostigmine. Similar beneficial effects were not observed with the central nervous stimulant methylphenidate. In fact, methylphenidate potentiated the behavioral impairment of scopolamine, at doses which normally produce no measurable effects. Earlier pilot work showed that higher doses of methylphenidate produced even greater impairments when given concurrently with scopolamine. Thus, it is very unlikely that any dose of methylphenidate could be expected to reverse the scopolamine-induced amnesia. Because the primary effects of methylphenidate are on the central nervous system (exerting relatively weak peripheral autonomic effects) [8], and the scopolamine-induced amnesia is central in nature [6,11], it is unlikely that other stimulants, even less specific to the central nervous system, would have fared any better. Recent research studying the effects of amphetamine on the scopolamine-induced amnesia in humans supports this contention [9].

Although not tested in the present experimental design, the results of other recent research indicates that the doses of physostigmine used (0.02–0.03 mg/kg), when delivered alone to the same young monkeys, do not produce the same consistent, positive effects as when reversing the effects of scopolamine [3]. These data suggest that the effects of physostigmine on memory are more easily measurable when reversing a specific, cholinergic dysfunction, than when given alone to improve presumably normal functioning. At the same time, the data provide additional support for the notion that the amnesia induced by scopolamine is most probably due to a specific disruption of important, memory-related cholinergic mechanisms. Previous studies performed in this same test situation indicate that this deficit is uniquely and qualitatively similar to that suffered naturally by aged rhesus monkeys [2,4], suggesting that an important relationship between normal aging and cholinergic malfunction may exist. Similar observations have been made with human patients as subjects [9, 10, 11, 13]. Furthermore, recent biochemical analyses of post-mortum brain from aged human patients has demonstrated a positive correlation between degree of change in cholinergic function and degree of senile dementia [17,20]. Thus, the data from the gerontological and pharmacological clinics and from our primate laboratory share remarkable consistency and point to an important cholinergic role in the specific memory impairment associated with old age.

A question that remains to be answered is why methylphenidate potentiated the debilitating effects of scopolamine. Although a definite answer to this question awaits further research, several alternative possibilities are nevertheless apparent. For example, it is possible that the stimulant effects of methylphenidate served to increase the monkeys' sensitivity to irrelevant interferring stimulation during the retention interval, resulting in a greater deficit in short-term memory. Other research has in fact shown that such increases in sensitivity to interference occur naturally in the aged monkey [23] and man [19], as well as in some monkeys given low doses of scopolamine (Bartus, unpublished observations). Another possibility (not mutually exclusive of the first) is that the enhancement of catecholamine activity that results from methylphenidate [12] causes a reciprocal reduction or depression in cholinergic activity. Others have reported that such a negatively correlated relationship between these two transmitter systems should in fact be expected [1, 16, 18]. Certainly, one cannot make a definitive evaluation of either of these two ideas on the basis of currently available data. However, future research directed toward resolving issues such as these should help define the nature of the scopolamine-induced amnesia and ultimately clarify its relationship to that occurring naturally in aged humans and non-human primates.

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