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Effects of Cholinergic Drugs on Delayed Match-To-Sample Performance of Rhesus Monkeys[1]

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PENETAR, D. M. AND J. H. McDONOUGH, JR. *Effects of cholinergic drugs on delayed match-to-sample performance of rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **19**(6) 963–967, 1983.—Three adult rhesus monkeys were trained to stable performance baselines on a delayed match-to-sample (DMS) procedure. Subject-initiated trials resulted in brief presentations of a sample color stimulus (red, green, or blue) with matching performance tested after retention intervals of 0, 4, 8, and 16 seconds. The effects of graded doses of atropine SO_4, benactyzine HCl and physostigmine salicylate on performance were studied. Only atropine produced a clear interaction between drug and retention interval with the greatest impairments being observed at the longest delays. Benactyzine only affected overall error rates, while physostigmine did not disrupt matching accuracy. The two highest doses of each compound produced reliable elevations in session times and drug-specific changes in the pattern of performance. The results with atropine confirm and extend previously reported work with scopolamine indicating a critical role for cholinergic mechanisms in short-term memory.

Delayed match-to-sample Short-term memory Atropine Benactyzine Physostigmine Primate behavior

A series of studies by Bartus and colleagues have demonstrated that anticholinergic compounds are capable of disrupting short-term memory (STM) in nonhuman primates, and have lent support to the theory that the cholinergic system is critically involved in normal memory function [1, 3, 4]. These studies have been primarily restricted to a single test procedure, a delayed-response task, and a single anticholinergic compound, scopolamine. Further support could be gained for the role of the cholinergic system in memory function, if it were demonstrated that other cholinergic compounds produce similar effects using another behavioral test which requires STM for successful task performance. The present experiment investigated the effects of two anticholinergic drugs, atropine and benactyzine, and the anticholinesterase compound physostigmine, on performance of a delayed match-to-sample (DMS) task by nonhuman primates. Atropine and benactyzine were chosen as representative anticholinergic drugs since they closely mimic the effects produced by scopolamine on differential reinforcement of low rate (DRL) performance in rhesus monkeys [16]. Physostigmine was tested in an effort to replicate reports that it can facilitate performance of tasks requiring recent memory [2].

METHOD

Subjects

Three experimentally naive adult male rhesus monkeys (Macaca mulatta) weighing between 6–9 kg served as subjects. They were individually housed in stainless steel primate cages (60 cm wide × 68 cm deep × 76 cm high) with ad lib access to water. Food, with the exception of rewards earned in the behavioral task, was restricted to a single supplemental feeding (Purina monkey chow and a slice of fruit) given at least one hour after the experimental session. This feeding regimen maintained the animals at 90–95% of free feeding body weight.

Apparatus

One wall of a standard housing cage was replaced with an aluminum intelligence panel. Mounted horizontally on the panel and equidistant from each other (39 cm above the cage floor) were three press plate manipulanda (BRS No. PPC-012), each of which could be transilluminated with a 12 stimulus in-line projector. Mounted below each press plate was a standard primate response lever (BRS No. PRL-002).

[1]The experiments reported here were conducted according to the *Guide for Care and Use of Laboratory Animals* (1978) as prepared by the Committee on Care and Use of Laboratory Animals, National Research Council, DHEW Publication No. (NIH) 80-23. The opinions and assertations contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

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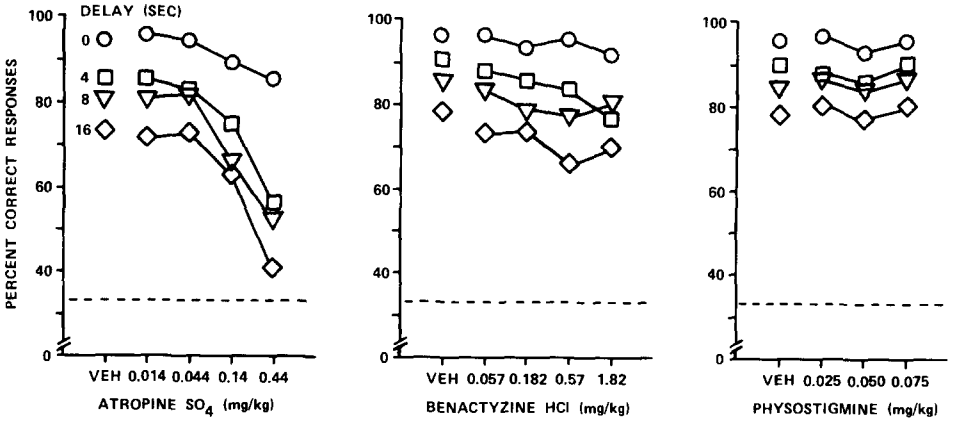

FIG. 1. The effects of atropine, benactyzine and physostigmine on mean percent correct responses at each MTS delay interval as a function of drug dose. Dashed line indicates chance levels of performance.

Centered above the three press plates was another in-line projector without response sensing capabilities which was used to present the sample stimulus. Banana flavored 300 mg food pellets (Noyes) served as reinforcing stimuli. A pellet dispenser (BRS No. PDC-040) dropped pellets into a food well centered just below the response levers. This testing cage was mounted inside a sound attenuating booth located in an experimental room which was flooded with masking noise. Programming and data recording equipment were located in a separate room.

Procedure

Subjects were trained to initiate a trial by responding on the center lever on a second-order Fixed Ratio (FR) schedule. Sample stimuli (colors of red, blue or green) were presented for 0.1 sec on the top backlit key after every two responses for a total of five times, FR5(FR2), per trial. Match stimuli appeared on the three push button backlit keys after delays of 0, 4, 8 or 16 sec. Center lever presses during the delay intervals were without consequence. Correct match responses were reinforced; incorrect match responses produced a five second time out (TO) in which all key lights and the house light were turned off. Responses during the TO reset the TO interval. Presentation order of sample colors, time delays and placement of match stimuli were randomized for each trial and a new set of conditions was generated for trials following incorrect matching responses. Session length was 200 reinforced trials. Data recorded were percent correct responses as a function of delay and session time.

Drugs

All drugs were administered intramuscularly in the lateral thigh. Benactyzine HCl (0.057, 0.182, 0.57, 1.82 mg/kg) and physostigmine salicylate (0.025, 0.050, 0.075 mg/kg) were administered 15 minutes prior to testing while atropine SO_4 (0.014, 0.044, 0.14, 0.44 mg/kg) was administered 45 minutes prior to testing. All doses are expressed as salts. The vehicle for all drugs was distilled water for injection, USP, with 0.5% methylparaben and 0.05% propylparaben (w/v) added for stabilization and adjusted to pH~2.6 with 0.1 N hydrochloric acid. All solutions were prepared so that injection volumes=0.1 ml/kg. The drugs were prepared in lots and stored under refrigeration between drug tests. Each dose of each drug was tested twice in each subject. Order of drug presentation and dosages given, including the vehicle control, were counterbalanced across subjects to control for order effects. Vehicle control injections were given at the appropriate delay time for the particular drug being tested. At least one week and four baseline sessions separated every test.

RESULTS

For each drug dose, the percentage of correct responses at each delay time was calculated and then for each drug, these data were analyzed by a dose × delay × replication analysis of variance. Time to complete each experimental session was also recorded and analyzed with a dose × replication analysis of variance for each drug. Reliable effects were evaluated further with Newman-Kuels and Dunnett tests [20].

Percent Correct Responses

There was no evidence of a replication effect with any of the three drugs, so these data were collapsed across replications. Figure 1 shows percent correct responses at each delay plotted as a function of dose.

The atropine data showed highly reliable effects for both dose, $F(4,8)=40.58$, $p<0.01$, and the dose × delay interaction, $F(12,24)=5.50$, $p<0.01$. For the dose effect, performances were ordered in the following manner: veh=0.014 mg/kg=0.044 mg/kg>0.14 mg/kg>0.44 mg/kg. Analysis of the dose × delay interaction indicated this factor totally accounted for the increased errors at the 0.14 and 0.44 mg/kg doses. In both cases there was a direct relationship between reduced matching performance and delay times. Percent correct responses were significantly reduced from control values at the 4, 8, and 16 second delays by the 0.44 mg/kg dose, while percent correct responses were reduced only at the 8 and 16 second delays with the 0.14 mg/kg dose. The 0.044 and 0.014 mg/kg doses did not alter performance compared to vehicle control sessions at any delay time. Analysis of the benactyzine data revealed that the effect of dose was marginally reliable, $F(4,8)=3.77$, $p=0.052$, while the dose × delay interaction was not. Both 0.57 and 1.82 mg/kg benactyzine reduced overall correct responses relative to vehicle

control performance (t's=3.00 and 3.32 respectively, ps<0.05). Physostigmine produced no reliable effects at any dose on matching performance.

Session Time

In contrast to error rates all drugs produced highly reliable effects on time to complete the session, atropine: $F(4,8)=21.94$, $p<0.01$; benactyzine: $F(4,8)=5.44$, $p<0.05$; physostigmine: $F(3,6)=45.01$, $p<0.01$. These data are displayed in Fig. 2.

Only the highest dose of atropine (0.44 mg/kg) and benactyzine (1.82 mg/kg) significantly increased session times, while both 0.050 and 0.075 mg/kg of physostigmine did. In addition, there was a reliable dose × replication interaction with atropine, $F(4,8)=4.11$, $p<0.05$. Subjects took significantly less time to complete the session the second time they received the 0.44 mg/kg dose of atropine ($\bar{x}$=263 min) than on the first test ($\bar{x}$=419 min), although both session times were significantly elevated over the control value.

One aspect of the results not readily apparent from the above data is the way in which these three compounds differentially affected response topography within the session. Figure 3 displays representative cumulative records which illustrate these effects. Baseline performance records display responding typical of FR behavior with subjects rapidly pressing 10 times for the 5 presentations of the sample stimuli on each trial. Performance was continuous and at approximately the same rate throughout the session. The two highest doses of physostigmine produced dose-related periods of nonresponding at the start of the sessions, yet once responding was initiated performance appeared normal. In contrast, animals receiving the high doses of atropine performed at a slower than normal rate more or less continuously throughout the session. Although there was some tendency for long (5–20 min) breaks in responding to occur during the 0.44 mg/kg dose sessions, matching accuracy remained poor throughout the session. With benactyzine errors tended to be clustered early in the session with animals displaying relatively normal performance as the session progressed. Also in a number of records, especially at the 1.82 mg/kg dose, there were initial periods of nonresponding as was observed with physostigmine.

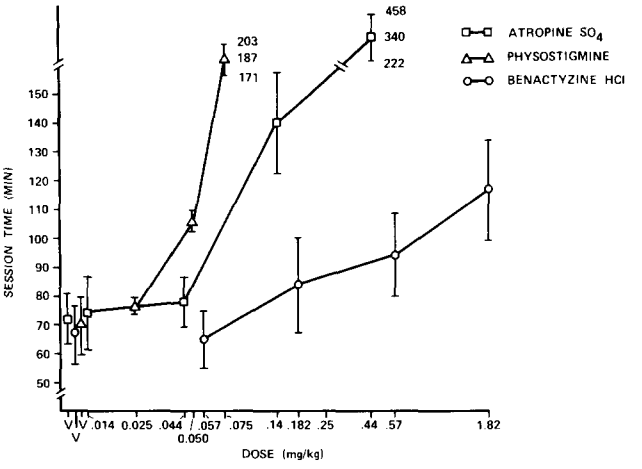

FIG. 2. The effects of atropine, benactyzine and physostigmine on mean time to complete the 200 reinforcement MTS session. Bars represent standard errors of the mean. Vehicle session times indicated by Vs.

DISCUSSION

The significant interaction between dose and length of retention interval provides rather strong evidence that the effect of atropine on DMS performance was due to disruption of STM mechanisms. As the amount of time information must be held in memory increased, the disruptive effects of atropine increased. Additionally, there was evidence for a dose-effects relationship in that low (0.14 mg/kg) doses affected only the longest retention intervals while the higher dose affected even shorter retention intervals and the performance at the longest interval approached chance levels. These results are similar to STM deficits produced by the anticholinergic drug scopolamine in monkeys performing on a delayed response task [1,3], and are in agreement with work in human subjects which indicates that anticholinergic drugs interfere specifically with the ability to store new information into STM [4, 8, 9, 17, 18].

The failure to produce decrements indicative of a specific STM deficit in MTS performance with benactyzine is surprising. The doses tested were well within the range reported to disrupt performance of rhesus monkeys working on a DRL schedule [16] or an equilibrium and multiple response task [11]. The fact that the higher doses of benactyzine reduced the percentage of correct responses and increased session times also indicates the drug exerted some disruptive effect on performance, yet the decrement was not nearly as severe as would be predicted based on the results of the two studies cited above. One reason for these seemingly reduced drug effects may be due to the relatively short time-course of benactyzine. Both human [6, 13, 14, 15] and animal studies [11,15] have demonstrated benactyzine has a rapid (≤15 min) onset of effect but a relatively brief (1–2 hr) time course. Given the delay between injection and testing (15 min), the tendency for the subject not to initiate trials for varying lengths of time after the start of the session, and the observation that performance became progressively better as a session progressed, it appears that peak drug effects may have occurred only for short portions of the session when the animal was actually performing. Therefore, several factors, specifically the long test session and the short acting nature of the drug, may have combined to alter the effective drug dose across time. This would mask any selective drug effect benactyzine may produce on STM at low doses, or artificially shift the dose-effect curve to the right if higher doses had been used. A more complete assessment of the effects of benactyzine on STM would require the analysis of performance by blocks of trials over the session or the use of a more condensed testing procedure with forced pacing of trials to capture the short time-course of this compound.

It is well established that anticholinergic drugs can affect visual discrimination performance [3,10] and it has been argued that anticholinergic-related deficits in some memory tasks may be due to a more general disruption of visual discrimination abilities rather than a specific effect on STM [3]. Several points argue this was not the case in the present study. Human studies indicate that these anticholinergic drugs disrupt visual acuity but not color vision or the ability to discriminate colors ([6]; Brown, personal communication). The prime reason for choosing colors to serve as stimuli in this study was to minimize visual acuity requirements for successful task performance. Secondly, the significant dose × delay interaction with atropine, along with the lack of

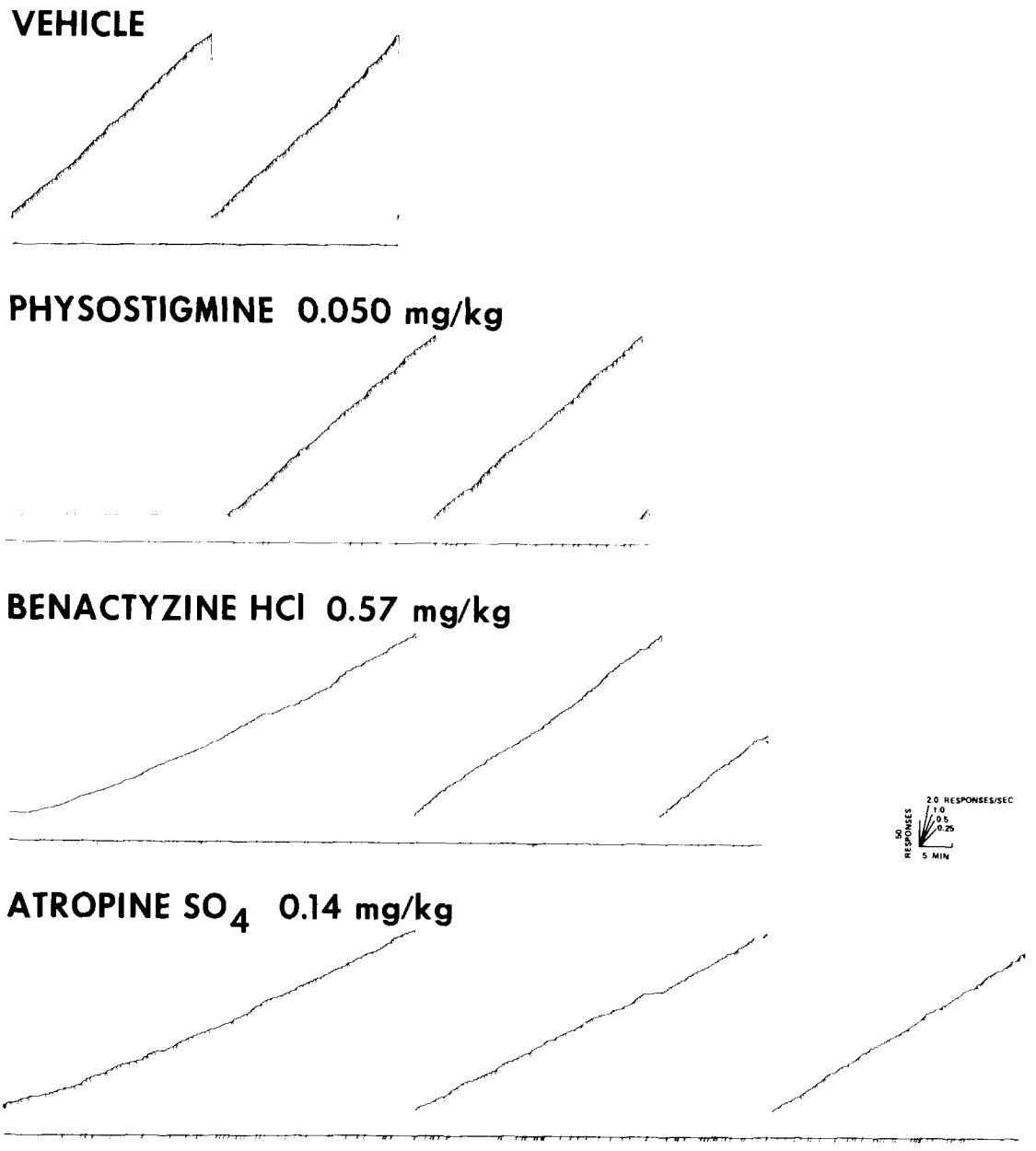

FIG. 3. Representative cumulative records of one subject under vehicle and the indicated drug conditions. Pen steps on each top trace indicate responses to present sample stimuli, and deflections indicate correct match responses; deflections of the event pen (lower trace) indicate incorrect matches. The recorder ran during delay intervals, but did not run during time-out after an incorrect match.

a matching impairment at the 0 sec delay, indicates the memory requirements of the task were particularly sensitive to the effects of the drug rather than discrimination ability. Even though there was no evidence for a specific STM deficit with benactyzine, the higher doses of this drug also increased errors only at the longer delay intervals but not at the 0 sec delay. Thus, the data indicate that neither drug significantly disrupts the ability to discriminate the color stimuli when the match choice was immediate. Only when delays were interposed was performance affected.

The finding that physostigmine did not affect STM function as measured by the DMS procedure does not support the results of Bartus [2] who has reported physostigmine can enhance delayed response performance in both young and old monkeys. The doses of physostigmine used in this study were chosen based on work using variable interval schedules of performance [7,19], and are relatively high compared to those studied by Bartus. He reported 0.020 mg/kg of physostigmine improved performance in half of both the young and old subjects [2], while the almost equivalent dose of 0.025 mg/kg in this study produced no consistent effects with any of the three subjects. Bartus did note however, that such effects were not large in magnitude, variable between subjects, and usually restricted to a narrow dose range which

was subject-specific. The data collected here also revealed a great deal of intra- and intersubject variability in response to physostigmine despite a highly consistent and replicable pattern of baseline performance. A given dose often produced apparent improvements and decrements within the same subject and these effects were not systematically related to retention interval. Additionally, apparent improved performance could not be consistently reproduced in the same subject. In only one case, at 0.075 mg/kg, did a subject show improvement in matching performance at all retention intervals on both drug runs. Thus, the results of the present study neither support nor refute findings that low doses of physostigmine can enhance STM performance. Several procedural variations may be required to more conclusively address this question using the present procedure, to include a larger number of subjects, longer delay times, and individually tailored drug doses.

In both the DMS procedure as used here and the delayed response procedure of Bartus [3] all trials are initiated by the subject. After high doses of drug—especially physostigmine—subjects did not initiate trials for varying periods of time. Yet, using other procedures (i.e., Sidman avoidance, fixed interval, FR, DRL) it has been noted that animals continue to perform tasks, albeit at lower rates or with decremented efficiency, at doses of anticholinergics or anticholinesterase even higher than those tested here [5, 7, 11, 12, 16]. It was observed here and in other studies in this laboratory that subjects will readily accept and consume food pellets during the periods of non-responding. This would seem to indicate that nonspecific drug effects on motor abilities or motivation are not always primary factors in the refusal of an animal to perform. Experiments with human subjects which test the effects of drugs on memory use procedures where subjects are not afforded the choice of simply not performing. Testing is usually rigidly time-locked to the time of drug administration. Testing procedures for assessment of drug induced changes of memory function in animals may possibly be improved by forced pacing of trial presentations. Shock avoidance contingencies could be arranged which would require an animal to initiate a new trial within a fixed period of time after responding on the previous trial to avoid shock. Such a procedure would be especially useful in testing drugs with short durations of action or which produce dose-related cessations in responding not accompanied by obvious motor impairment.

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