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Acute Effects of Physostigmine on Complex Operant Behavior in Rhesus Monkeys

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FREDERICK, D. L., G. E. SCHULZE, M. P. GILLAM AND M. G. PAULE. *Acute effects of physostigmine on complex operant behavior in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **50**(4) 641-648, 1995.—The effects of physostigmine were assessed in rhesus macaques using behavior in several complex tasks designed to model aspects of time estimation [temporal response differentiation (TRD)], short-term memory [delayed matching-to-sample (DMTS)], motivation [progressive ratio (PR)], learning [incremental repeated acquisition (IRA)], and color and position discrimination [conditioned position responding (CPR)]. The endpoints monitored included percent task completed, response rate, and accuracy. Physostigmine sulphate (0.001-0.056 mg/kg) significantly decreased the percentage of task completed and response rate in each task at 0.03 and 0.056 mg/kg. Accuracy in the TRD task was significantly decreased at 0.03 and 0.056 mg/kg, whereas accuracy in the CPR and IRA tasks was significantly decreased only at 0.056 mg/kg. DMTS accuracy was not significantly affected at any dose tested. A significant increase in accuracy was noted in learning task performance at the 0.01 mg/kg dose, although only for one-lever response sequences. Performance enhancements were not seen in any other task. These results indicate that in monkeys, low doses of physostigmine may facilitate acquisition or learning of simple one-lever spatial tasks while not significantly altering the acquisition of similar but more complex tasks. Impaired task performance at high doses may be more reflective of cholinomimetic side effects (tremor and hypothermia) that affect response rate than a central or "cognitive" impairment.

Monkeys Physostigmine Operant behavior Learning Incremental repeated acquisition Memory Delayed matching-to-sample Time perception Temporal response differentiation Motivation Progressive ratio Color and position discrimination Conditioned position responding

PHYSOSTIGMINE, an alkaloid purified from the calabar bean (*Physostigma venenosum*), is a prototypic reversible anticholinesterase agent. Physostigmine has been extensively used in studying the effects of acetylcholinesterase inhibition, and thus enhanced cholinergic neurotransmission at both nicotinic and muscarinic receptor sites (38). Because of its tertiary structure, physostigmine crosses the blood-brain barrier, producing central as well as peripheral effects. The primary clinical use of physostigmine is in the treatment of glaucoma and in the antidotal treatment of intoxication with anticholinergic compounds (19,38).

The behavioral effects of physostigmine are well documented and include dose-dependent decreases in locomotor activity (30), increases and decreases (depending on the dose and baseline response rate) in rates of responding for food reinforcement (5,11,28), and both facilitation and disruption of cognitive performance (1,2,5,12,13,18,28,40). Central cholinergic systems, which have long been implicated in the modulation of cognitive functions such as learning and memory, have received considerable experimental attention in both animals and humans (3,4,14,17,41). For example, muscarinic receptor blockade has been associated with decrements in performance of tasks designed to measure learning ability and memory retention (28,29,31), whereas cholinergic stimulation has been shown to facilitate performance of similar tasks in a variety of animal species, including humans (2,5,7,12). In

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addition, decrements in central cholinergic neurons have been implicated in human memory disorders and Alzheimers disease (6).

There is some controversy surrounding the reported ability of physostigmine to facilitate performance of learning and memory tasks (8,28,29,37). When facilitation has been reported, the effects were not large, were quite variable between subjects, and were restricted to a narrow, subject-specific dose range (5,29).

Most investigations concerning the behavioral effects of physostigmine have focused on a single behavior rather than on a battery of different behaviors. In this laboratory, the neurobehavioral effects of a number of psychotropic compounds have been evaluated using performance by monkeys in an operant test battery (OTB) [see (22) for an overview]. The OTB was devised to permit the simultaneous assessment of multiple behaviors believed to model different complex brain functions. This approach allows determination of the relative sensitivities of the different behaviors to disruption by a particular drug or toxicant. In addition, OTB performance of well-trained rhesus monkeys is generally indistinguishable from that of human children (25) and performance of children in the OTB correlates well with traditional intelligence quotient measures in the same subject (24). The tasks and the brain functions they are thought to model include temporal response differentiation (TRD; time estimation), delayed matching-to-sample (DMTS; short-term memory), progressive ratio (PR; motivation to work for food), incremental repeated acquisition (IRA; learning), and conditioned position responding (CPR; color and position discrimination).

The present experiment was one of several studies designed to validate the use of the OTB to assess neurobehavioral toxicity through testing of relatively well-characterized, reversibly acting drugs as reference compounds in monkeys. Physostigmine doses (0.001–0.056 mg/kg) were chosen based on literature reports and the criteria that the highest dose tested grossly affected most behavioral endpoints, and the lowest dose was without detectable effects. Physostigmine was chosen for study because of the reversibility of its effects after acute administration and its relatively well-characterized mechanism of action (38), and to complement our recent experiments with the prototypic cholinergic muscarinic antagonist atropine sulfate (31).

METHOD

Subjects

Seven male rhesus monkeys (*Macaca mulatta*), 4–7 years of age,weighing 4–9 kg at the beginning of the study, served as subjects. All animals had been previously trained to perform the tasks in the OTB for several years and had been used in previous studies on the acute effects of several psychoactive compounds (31–36), during which a minimum of 1 month separated each drug exposure. In all previous acute drug studies (31–36), the maximum dose administered never exceeded a toxic level, and no residual effects were noted in the baseline OTB performance of any monkey after such acute exposure. Animal housing, feeding, and so forth were as described previously (32). Briefly, each monkey was individually housed and fed its daily allotment of food (Purina Hi Protein Monkey Chow; Ralston Purina, St. Louis, MO) supplemented with fresh fruit and chewable multivitamins with iron (Arkansas Cooperative Assoc. Inc., North Little Rock, AR) after each test session. Water was available ad lib. Animal care and procedures were in accordance with the American Association for Accreditation of Laboratory Animal Care (AAALAC) guidelines and approved by the NCTR Institutional Animal Care and Use Committee.

Apparatus

The apparatus consisted of portable restraint chairs, sound-attenuated behavioral chambers, operant panels, and computer consoles, which have been previously described (32). The operant panels were equipped with three rear-projection press-plates, four retractable levers, six serial position indicator lights, and correct and incorrect response indicator lights. The press-plates, levers, and indicator lights were aligned horizontally, with the press-plates and serial position indicator lights located above the levers. Symbols and colors were projected onto the press-plates from the rear. When operated, both levers and press-plates effected a switch closure. Serial position and correct and incorrect indicator lights were illuminated from behind the panel with various colors. A trough for reinforcer (190-mg banana-flavored food pellet) delivery was centered below the levers.

Operant Schedules

A brief description of the operant tasks contained in the OTB follows. The use and description of the tasks contained in the OTB have also been reported in detail elsewhere (22,32), and a diagram of the behavioral test panel is shown in Paule et al. (27).

Time estimation task (TRD). Only the left of the four levers was extended and active. Subjects were required to hold the lever in the depressed position for a minimum of 10 s but not longer than 14 s. Releasing the lever within the 4-s window resulted in reinforcer delivery. Releasing the lever too early or too late ended the current trial, after which the monkey could immediately start another trial.

Short-term memory task (DMTS). Only the three press-plates were used (levers were retracted). At the start of each trial, one of seven geometric symbols (the "sample") was projected onto the center plate in a random fashion (side press-plates were dark). To continue the trial, each monkey was required to make an "observing" response (a press) to the center plate. After the observing response was made, the center plate was extinguished for one of six possible time delays, presented pseudorandomly. Of the five animals showing stable performance in this task, each was presented time delays of 2, 8, 16, 32, 48, and 64 s, during which all three press-plates were dark. After the time delay, all three plates were illuminated, each with a different geometric symbol, only one of which matched the sample. A response to the "match" resulted in reinforcer delivery and initiation of a new trial with another sample stimulus (presented randomly). A nonmatching response was followed by a 10-s time-out period (all plates darkened) and then initiation of a new trial.

Motivation task (PR). Only the far-right retractable lever was extended and active. Each monkey was required to increase the number of lever presses required for each subsequent reinforcer. Initially, one or two lever presses (depending on the individual monkey but the same for each subject every test day) resulted in reinforcer delivery. The number of responses required for the next reinforcer was increased by the initial number of lever presses required for the first reinforcer. Thus, if two lever presses were required for the initial reinforcer, four lever presses were required for the next, then six, eight, and so on. The ratio increments were chosen so that

marked periods of pausing or cessation of responding generally occurred during each baseline or vehicle PR session.

Learning task (IRA). All four retractable levers were extended and the serial position and correct and incorrect response indicator lights were used. Subjects were required to learn or acquire a new sequence of lever presses each test session. The IRA task began with the presentation of a one-lever sequence (IRA1). Each response on the correct one of the four levers resulted in reinforcer delivery. After 20 correct, but not necessarily consecutive, response sequences (criterion performance), a 1-min time-out period was followed by the presentation of an "incremented" two-lever sequence (IRA2) in which a response on a different lever was required before a response on the original (IRA1) lever produced a reinforcer. After 20 errorless two-lever sequences (i.e., no errors were made between the first and last correct lever presses of the required sequence), the task was incremented to a three-lever sequence and so on, up to a six-lever sequence or until the allotted task time had elapsed. The serial position indicator lights signalled position in the response sequence, indicating the remaining number of correct responses necessary for reinforcer delivery. Incorrect responses were followed by a 2-s time-out (illumination of the incorrect response indicator light) but did not reset the response requirement; thus, error correction was permitted. Correct responses were followed by illumination of the appropriate serial position indicator light and a 1-s time-out with illumination of the correct response indicator light.

Color and position discrimination task (CPR). Only the three press-plates were used (levers were retracted). At the start of each trial, the center plate was illuminated with either a solid red, yellow, blue, or green color (side press-plates were dark). Subjects continued the trial by making an observing response (a press) to the center plate, after which it was extinguished and the two side plates were immediately illuminated white. If the center plate color had been either blue or green, a response to the right press-plate (white) resulted in reinforcer delivery and initiation of a new trial. If the center press-plate had been either red or yellow, a response to the left press-plate (white) resulted in reinforcer delivery and initiation of a new trial. Responding to the incorrect position initiated a 10-s time-out period followed by the initiation of a new trial. The sequence of color presentation was random.

Behavioral Testing Procedure

Behavioral test sessions were conducted daily (Monday through Friday) and lasted approximately 50 min. Monkeys were rotated through nine identical behavioral test chambers so that, in general, no monkey was placed in the same chamber on two consecutive test days. Behavioral schedules alternated daily. For example, PR (10 min), IRA (35 min), and CPR (5 min) were presented on one test day; TRD (20 min) and DMTS (30 min) were presented the next test day.

Drugs and Dosing Procedure

Physostigmine hemisulfate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile bacteriostatic (0.9% benzyl alcohol) saline (Elkins-Sinn Inc, Cherry Hill, NJ) for an injection volume of 0.1 ml/kg. The purity of the physostigmine was determined to be 99.5% by in-house HPLC analysis using an ultraviolet detector set at 248 nm. Doses of physostigmine [0.00, 0.001, 0.003, 0.01, 0.03 and 0.056 mg/kg, intravenously (IV)] were administered in a randomized order. Because of the daily alternation of behavioral tasks, all doses were given twice to provide dose–response data for each set of operant tasks. Generally, physostigmine injections were given on Tuesdays and Fridays, whereas saline injections were given on Thursdays. Approximately 15 min following injections, subjects were placed into operant chambers, and behavioral sessions began 1 min later.

Behavioral Endpoints

The end points measured in each task have been described in detail elsewhere (32). Three fundamental measures were monitored for most tasks: percent task completed (PTC), response rate or latency, and response accuracy.

PTC. The PTC data are measures of a predetermined performance criteria and are functions of both response rate and response accuracy. The PTC measure is calculated by dividing the total number of reinforcers earned in a given session by the total number of reinforcers possible and multiplying this quotient by 100. The total number of reinforcers possible for a given task was chosen arbitrarily based on the length and difficulty of the task. The PTC endpoint is a convenient and comprehensive measure showing intra-animal stability, and has proven useful for comparing drug effects on performance across tasks.

Response rate and response latency. Response rate for each of the PR and TRD tasks were calculated by dividing the total number of lever presses by the total session time (in seconds). Response rate for each of the CPR, DMTS, and IRA tasks were calculated by dividing the total number of responses by the total session time minus time-out and delay periods (in seconds). For the DMTS and CPR tasks, mean response latencies were also calculated for both observing and choice responses. If a monkey did not make an observing and/or choice response, a maximum response latency of 300 s was used in the analyses. In addition to overall response rate for the IRA task (collapsed across components), response rates were measured for individual components or levels within the IRA task.

Response accuracy. Response accuracy for each of the CPR and DMTS tasks was calculated by dividing the number of correct responses by the total number of trials in a given session and multiplying this quotient by 100. For the TRD and IRA tasks, response accuracy was calculated by dividing the total number of correct lever presses by the total number of lever presses in a given session and then multiplying this quotient by 100. Response accuracy is not applicable for the PR task.

Other measures. For the TRD task, mean duration of lever hold, and for the PR task, the breakpoint (the magnitude of the last ratio completed for which the monkey earned a reinforcer) were also calculated. For the IRA task, between-sequence errors (those occurring before the first correct response in a given sequence) and within-sequence errors (those occurring between the first and last correct response of a given sequence) were also recorded.

Statistical Analysis

Only those monkeys exhibiting stable performance for the measure of percent task completed after saline (vehicle) injections were included in the statistical analysis. Stable performance was defined as that having a standard error of $< 15\%$ of the mean for the vehicle sessions. During this study, all seven animals exhibited stable preexposure baselines for the TRD, PR, IRA, and CPR tasks, and five exhibited stable baselines for the DMTS task. For a subject's data to be in-

cluded in the TRD and CPR accuracy analyses, a minimum of three trials had to be completed. For inclusion in the DMTS and IRA accuracy analyses, a monkey had to complete a minimum of 10 trials. For DMTS group accuracy data presented by time delay, significance was assigned to those means falling outside the 95% confidence intervals constructed from vehicle control observations. The overall effect of drug treatments on performance in the various tasks was determined using a one-way repeated measures analysis of variance. When overall significance was evident ($p < 0.05$), then performance at each dose was compared to vehicle control performance by Bonferroni's (BON) multiple t-tests (21), which is generally considered to be a more conservative post-ANOVA test than either the Duncan or the Dunnett test.

RESULTS

Results from the five OTB tasks are summarized in Table 1. Baseline (noninjection) data were not significantly different from those for saline vehicle injections for any of the behavioral endpoints monitored (not shown). In Table 1 and for all subsequent references, "overall" refers to data collapsed across all time delays in the DMTS task and across all levels in the IRA task.

TRD

Under vehicle conditions, the TRD schedule generated average response rates of 0.14/s, response accuracies averaging 29%, and percent task completed values of 33% (120 reinforcers possible). Compared to vehicle controls, physostigmine significantly decreased TRD response rate, accuracy, and PTC at 0.03 and 0.056 mg/kg. The frequency of lever holds that were 2 s or longer in duration are shown in Fig. 1. Response bursts (lever holds of < 2 s), which are common in the TRD task, are shown in Fig. 2. Mean duration of lever

TABLE 1

DOSE OF PHYSOSTIGMINE (in mg/kg)

Task	End point	0.001	0.003	0.01	0.03	0.056
Time estimation	PC	–	–	–	*	*
(TRD)	RR	–	–	–	*	*
	ACC	–	–	–	(5)*	(2)*
	Avg. hold	–	–	–	–	*
Short-term memory	PTC	–	–	–	–	*
and attention	Overall RR	–	–	–	*	*
(DMTS)	Observing RL	–	–	–	*	*
	Choice RL	–	–	–	–	–
	ACC	–	–	–	–(4)	–(1)
Motivation (PR)	PTC	–	–	–	*	*
	Breakpoint	–	–	–	*	*
	RR	–	–	–	*	*
Learning (IRA)	PTC	–	–	–	*	*
	Overall RR	–	–	–	*	*
	ACC	–	–	–	–(5)	(2)*
Color and position	PTC	–	–	–	*	*
discrimination	RR	–	–	–	*	*
(CPR)	Observing RL	–	–	–	–	*
	Choice RL	–	–	–	*	*
	ACC	–	–	–	–(4)	(3)*

*Significant difference from vehicle (saline) performance. DMTS task ($N = 5$); $N = 7$ in all other tasks unless otherwise noted (parentheses). PTC = percent task completed; RR = response rate; RL = response latency; ACC = accuracy.

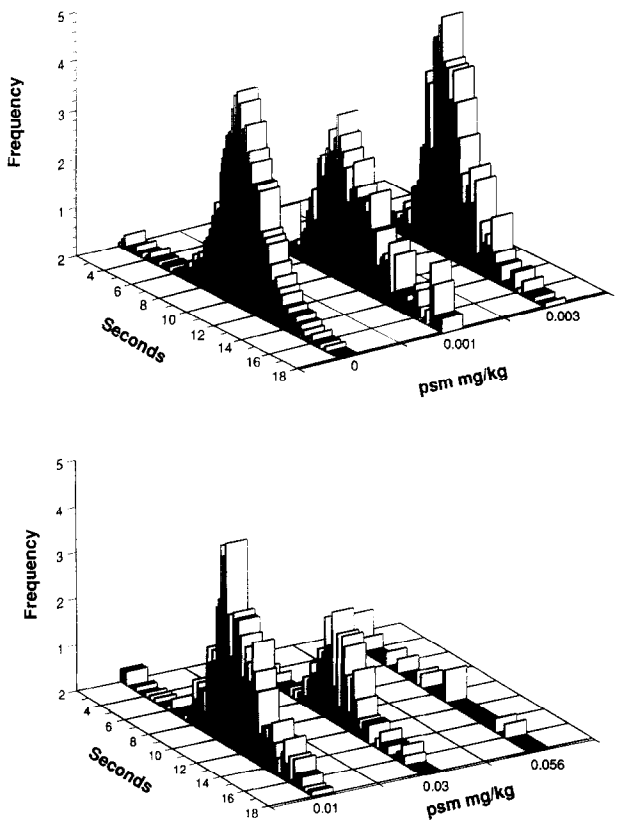

FIG. 1. (A and B): Effect of physostigmine on duration of lever hold in the temporal response differentiation (TRD) task for holds ≥ 2 s. Data are means for all seven subjects. Bars represent 0.2-s intervals (i.e., the first bar represents the frequency of lever holds with a duration of 2.00–2.20 s).

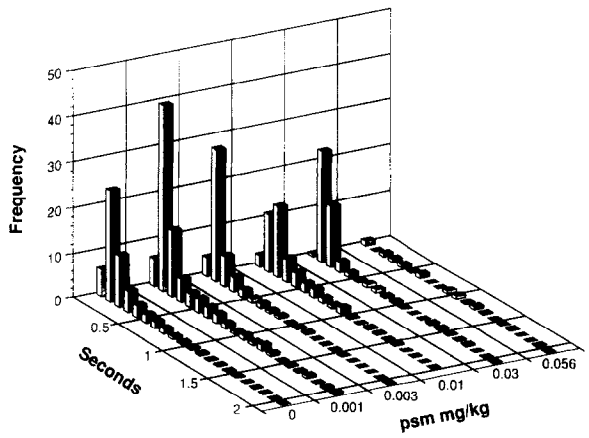

FIG. 2. Effect of physostigmine on the duration of lever hold in the temporal response differentiation (TRD) task for holds < 2 s. Data are as described in Fig. 1, except that bars represent 0.1-s intervals (i.e., the first bar represents lever holds with a duration of 0.00–0.09 s).

hold (all lever presses considered) was significantly affected only at the 0.056 mg/kg dose.

DMTS

Under vehicle conditions, the DMTS schedule generated overall response rates averaging 0.28/s, observing response latencies averaging 4.7 s, choice response latencies averaging 0.28 s, response accuracies averaging 73%, and percent task completed values of 42% (120 reinforcers possible). Physostigmine significantly decreased DMTS PTC at 0.056 mg/kg. Significant increases in mean observing response latencies occurred at 0.03 and 0.056 mg/kg. No statistically significant increases or decreases in choice response latencies occurred at any dose tested; however, the overall response rate at the 0.03 and 0.56 mg/kg doses was nearly completely suppressed in all subjects, resulting from the observation that very few (or no) choice responses were made at these doses. Hence, choice response latency was not an applicable measure for the 0.03 and 0.056 mg/kg doses. Although no statistically significant drug-related effects were observed in the accuracy of responding in the DMTS task (Fig. 3), the profound suppression of overall response rate at the higher doses resulted in only one subject completing the necessary 10 trials for inclusion in the accuracy measure at the 0.056 mg/kg dose (not shown), and just three reached criteria for inclusion at the 0.03 mg/kg dose.

PR

Under vehicle conditions, the PR schedule generated average response rates of 2.0/s and breakpoints of 96 responses (i.e., the size of last ratio completed that resulted in reinforcer delivery). Physostigmine significantly decreased PR response rate, PTC, and breakpoints at doses of 0.03 and 0.056 mg/kg. Figure 4 shows average interresponse time distributions obtained across PR sessions and illustrates the marked decrease in response frequency at doses > 0.01 mg/kg, and the virtual lack of effect at doses up to 0.01 mg/kg.

IRA

Under vehicle conditions, the IRA schedule generated average overall response rates of 1.6/s, which were roughly equivalent to those obtained for the two-lever sequence, the IRA2 component. Overall response accuracies averaged 59% (62% for IRA-2), and percent task completed values were 66% (120 reinforcers possible). Physostigmine significantly decreased IRA PTC and overall response rate at doses of 0.03 and 0.056 mg/kg (no subject was able to complete the 20 errorless sequences necessary to advance beyond IRA1 at this dose). A significant increase in response accuracy was detected at the 0.01 mg/kg dose for the one-lever sequence (IRA1; Fig. 5a and b), an effect not seen at any of the longer response sequences (not shown). The effects of physostigmine on within- and between-sequence errors for the IRA task at the two-lever sequence (IRA2) are shown in Fig. 6.

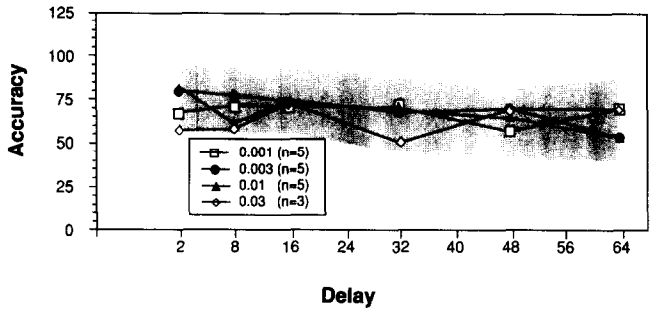

FIG. 3. Effect of physostigmine on delayed matching-to-sample (DMTS) response accuracy. Data are means for all five subjects unless otherwise noted. The shaded area represents the 95% confidence interval constructed from data for vehicle control sessions. The highest dose tested (0.056 mg/kg) abolished responding in all but one subject and was omitted.

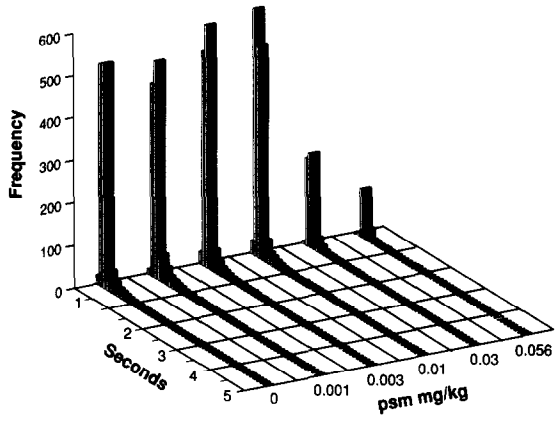

FIG. 4. Interresponse time distributions for the progressive ratio (PR) task. Data are means for all seven subjects. Physostigmine produced a significant dose-dependent decrease in all other PR endpoints measured (data not shown).

CPR

Under vehicle conditions, the CPR schedule generated average response rates of 0.7/s, observing response latencies of 2.4 s, choice response latencies of 0.25 s, response accuracies averaging 96%, and percent task completed values of 96% (60 reinforcers possible). Physostigmine significantly decreased CPR PTC and response rates at doses of 0.03 and 0.056 mg/kg. Response accuracy was significantly decreased at 0.056 mg/kg. Observing response latencies were significantly elevated at the 0.056 mg/kg dose, whereas choice response latencies were significantly elevated at both the 0.03 and 0.056 mg/kg doses. Figure 7 shows mean interresponse time distributions and illustrates the marked decrease in response frequency at doses of 0.03 mg/kg and above, and the lack of effect at doses ≤ 0.01 mg/kg.

DISCUSSION

In the current experiment, physostigmine produced a significant, dose-dependent decrease in response rate or latency (particularly at the two highest doses tested) in each OTB task, resulting in a decrease in the number of reinforcers earned (percent task completed) across all tasks. Response accuracies, however, were differentially affected across tasks. Other endpoints monitored (such as duration of lever hold in the TRD task, and breakpoint in the PR task) were also affected in a dose-dependent manner. Using the occurrence of a significant disruption in task performance at doses lower than those affecting other tasks as a criteria for determining relative task sensitivity, tasks designed to model time estimation (TRD),

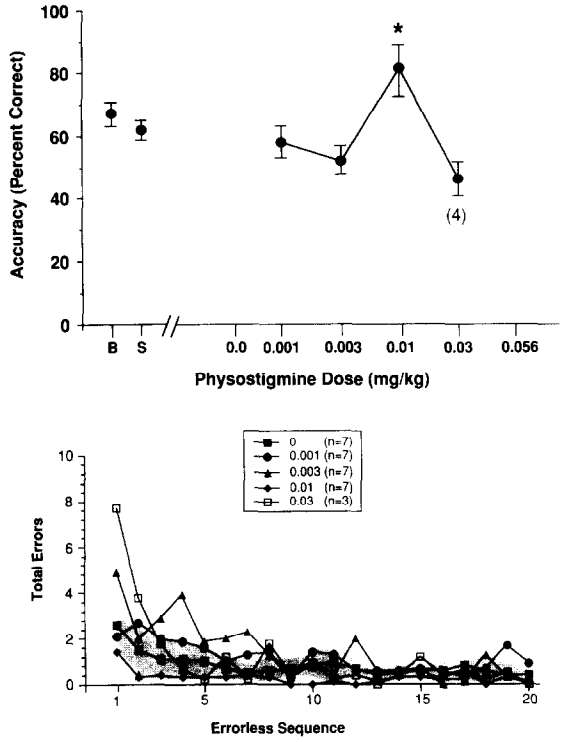

FIG. 5. Effect of physostigmine on accuracy (A) and total errors (B) in the incremental repeated acquisition (IRA) task at the initial one-lever (IRA1) sequence. Data are means for all seven subjects unless otherwise noted. At 0.056 mg/kg no subjects completed 20 errorless sequences at IRA1; therefore, there are no data shown for this dose. The shaded area represents the 95% confidence interval constructed from data for vehicle control sessions. Note the low number of total errors (B) at the 0.01 mg/kg dose compared to other doses.

short-term memory (DMTS), motivation (PR), learning, (IRA), and color and position discrimination were equally sensitive to the acute effects of physostigmine. The order of task sensitivity (TRD = DMTS = PR = IRA = CPR) obtained for physostigmine was distinguishable from a number of other drugs tested in this laboratory under similar conditions, including atropine (31), caffeine (10), cocaine (26), *d*-amphetamine (34), Δ-9-tetra-hydrocannabinol (32), diazepam (36), marijuana smoke (33), MDMA (unpublished results), MK-801 (23), morphine (35), nicotine (unpublished results), pentobarbital (16), and phencyclidine (23).

The profile of task sensitivity generated for physostigmine was identical to the profile previously generated for the dopamine antagonist chlorpromazine (15). The acute effects of both drugs on OTB task performance was qualitatively similar (although not identical) in that each significantly decreased response rate and PTC while differentially affecting response accuracy. For example, although DMTS accuracy was generally unaffected by either chlorpromazine or physostigmine at doses that disrupted response rate and PTC in this task, CPR accuracy was unaffected by equivalent doses of chlorpromazine, whereas physostigmine decreased CPR accuracy at the highest dose tested. The effects of physostigmine on DMTS accuracy in the present study are also consistent with other studies examining the effects of physostigmine on DMTS performance in rhesus (29) and squirrel monkeys (20).

In the last several years, the use of animal models to assess cholinergic involvement in the modulation of cognitive functions (such as learning and memory) has received increased experimental attention, due in part to the hypothesis that memory dysfunction is related to disruptions in cholinergic neural transmission. It is possible here to compare and contrast the acute effects of physostigmine (a prototypic reversibly acting cholinesterase inhibitor) used in the present study and atropine (a prototypic cholinergic muscarinic antagonist), the effects of which have been previously assessed in this laboratory using the OTB (31). A comparison of neurobehavioral profiles demonstrates few similarities; the profile of task sensitivity generated for physostigmine (TRD = DMTS = PR = IRA = CPR) differs greatly from that for atropine (IRA = CPR > TRD = DMTS = PR), a finding that is not unexpected, considering that these compounds have opposing effects on cholinergic transmission. A relatively low dose of physostigmine facilitated acquisition of the one-lever (IRA1) task but had little effect on overall accuracy, whereas atropine disrupted performance of this task at doses that generally did not affect performance of the other tasks. The PR task was highly sensitive to physostigmine's effects and one of the least sensitive to the effects of atropine.

Previous attempts to assess physostigmine's ability to enhance learning and memory (presumably by facilitating cholinergic transmission) have generally relied on performance in only a single operant task, and the results have been mixed and variable (5,28). If learning and memory were actually facilitated by physostigmine [as has been reported elsewhere (5,12,18,40)], it seems likely that performance of one or more OTB tasks would also be enhanced. In the present experiment, a significant improvement in response accuracy was noted in the IRA task for the initial one-lever sequence (IRA1) at 0.01 mg/kg. This effect was not seen at the longer response sequences (IRA2-6) or at any other dose tested, and there was no indication that physostigmine enhanced any aspect of performance in any other OTB task. It should be noted that response accuracy in the CPR task averaged 96% under vehi-

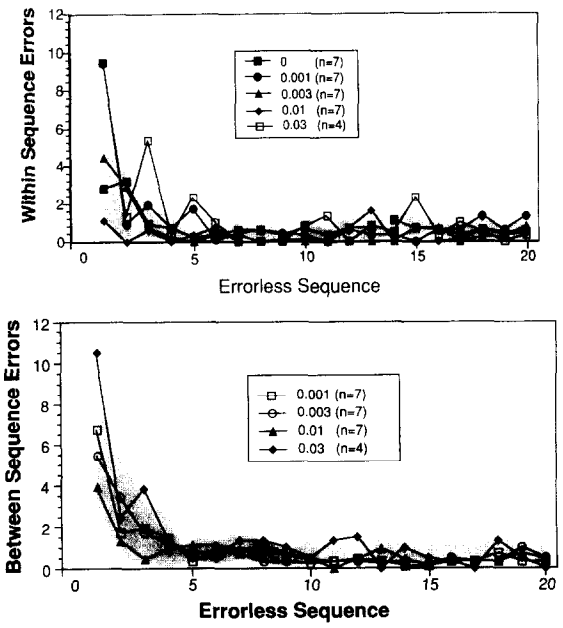

FIG. 6. Effect of physostigmine on within-sequence (top panel) and between-sequence (bottom panel) errors in the incremental repeated acquisition (IRA) task at the two-lever sequence (IRA2). Data are means for all seven subjects.

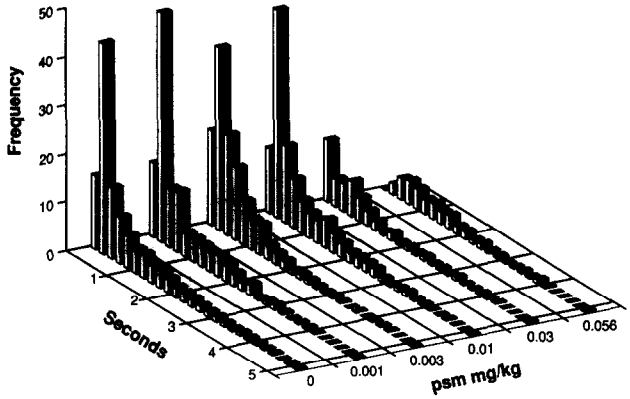

FIG. 7. Interresponse time distributions for the conditioned position responding (CPR) task. Data are means for all seven subjects. Note that although physostigmine significantly depressed CPR PTC (not shown) and response rate at 0.03 and 0.056 mg/kg, response accuracy was decreased only at 0.056 mg/kg (not shown), when responding was nearly abolished.

cle conditions; thus, any enhancement of the accuracy of performance in this task would not have been detectable because of a ceiling effect. These results indicate that in monkeys, low doses of physostigmine may facilitate the acquisition of simple one-lever spatial tasks, but that acquisition of more complex multilever tasks is not enhanced by physostigmine.

It is probable that the impaired OTB task performance noted in the present experiment reflects physostigmine's effects to decrease response rate rather than impair "cognitive" function. This interpretation is supported by a number of observations. For instance, response rate in the IRA and CPR tasks was significantly depressed at the 0.030 and 0.056 mg/kg doses, whereas response accuracy in these tasks was significantly decreased only at the higher dose. Also, DMTS accuracy was not significantly decreased at any dose, even though response rate in this task was significantly depressed at the two highest doses tested. If response rate for all tasks (excluding that for the time estimation task, which requires subjects to depress and hold down a response lever for a relatively long period of time) is considered, the order of task sensitivity to disruption by acute physostigmine is directly related to vehicle session (control) response rates. The most sensitive task (PR) also had the highest response rate during vehicle sessions (2.0 responses/s). Vehicle session response rates in the IRA and CPR tasks (which were less sensitive than those in the PR task) were 1.6 and 0.7 responses/s, respectively. The least sensitive task (DMTS) had the lowest vehicle session response rate of 0.28 responses/s.

Examination of within- and between-sequence errors in the IRA task also suggests that disrupted OTB task performance may have been the result of physostigmine's effect on response rate. Within-sequence errors occur when the subjects exhibit difficulty in recalling or performing the previously learned sequence, and are thought to reflect a relatively short-term memory impairment. A high number of between-sequence errors indicates that the subjects are having difficulty learning or acquiring the new or incremented sequence of lever presses, or that response perseveration is a prominent drug effect. In the present experiment, a low number of within- and between-sequence errors occurred in the IRA task, and there was no significant differences between the two error types (Fig. 6). This observation is indicative of nonspecific effects and suggests that disruptions in IRA performance were more likely due to a "noncognitive" effect of the drug. Penetar (28) also reported that physostigmine produced a similar effect in cynomolgus monkeys performing repeated acquisition tasks.

Several recent studies have noted that physostigmine produces significant cholinomimetic side effects (such as miosis, hypersalivation, hypothermia, and tremor) that are not related to its ability to influence cognitive function. Yoshida and Suzuki (42) demonstrated that although the cholinesterase inhibitors physostigmine, tacrine, and NIK-247 each reversed scopolamine-induced amnesia in rats at 0.03, 0.3, and 0.1 mg/kg respectively, physostigmine produced hypothermia and tremor at doses > 0.3 mg/kg, whereas 30 mg/kg of NIK-247 was required to produce similar effects. They concluded that NIK-247 had a higher safety factor and greater selectivity for cognitive functions than did physostigmine. It has also been reported that short-term memory processes in primates are enhanced (as evidenced by performance in DMTS tasks) by the combination of physostigmine and the central α-2 adrenergic agonist clonidine more than by administration of either drug alone. This combination permits the use of significantly higher doses of physostigmine, which was suggested as the possible explanation for such results (9,39).

In summary, the anticholinesterase physostigmine produced a neurobehavioral profile that was qualitatively similar to the profile previously generated for the dopamine antagonist chlorpromazine (15), but differed considerably from the profile for the muscarinic antagonist atropine (31), and profiles of other agents assessed using monkey performance in the OTB (22). The present results indicate that physostigmine may facilitate acquisition of simple spatial tasks at low doses, but that acquisition of more complex tasks is not affected or is disrupted by physostigmine. It is likely that the impaired OTB performance caused by the acute administration of physostigmine was primarily due to the drug's ability to suppress response rate rather than to affect cognitive processes. Such an interpretation is supported by the results of previous reports (9,39,42). Whether the performance-enhancing effects of physostigmine could be sustained during a chronic regimen is unknown.

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