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Effects of Physostigmine on Operant Serial Discrimination/Reversal Learning in Rats[1]

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CLISSOLD, D. B. AND G. A. HEISE. *Effects of physostigmine on operant serial discrimination/reversal learning in rats.* PHARMACOL BIOCHEM BEHAV **37**(1) 155–160, 1990.—Two experiments examined the effects of physostigmine on acquisition and performance of operant serial reversals by rats. In Experiment 1, four groups of rats (n = 6/group) were injected with either vehicle or 0.03 mg/kg physostigmine five minutes prior to each session, or vehicle or 0.5 mg/kg physostigmine immediately after each session of a three-stimulus (bright, dim or flashing light) repeated discrimination/reversal procedure. Rats treated with physostigmine pre- or postsession learned significantly more reversals over 50 sessions than animals injected with vehicle. Experiment 2 used only two discriminative stimuli, a light and a 2,500 Hz tone. Following establishment of a stable daily reversal baseline, postsession injections of physostigmine significantly increased the number of trials to criterion on the next session compared to each subject's control baseline. Results are attributed to enhanced between-session transfer of previously learned discriminated instrumental responses by physostigmine-treated animals.

Physostigmine Memory Reversal learning Serial discrimination Acetylcholine

THE cholinergic system has been implicated in learning and memory processes in both animals and in humans (8, 11, 12, 17, 24, 25, 37). More recently it has been suggested that cholinergic deficits may contribute to age-related memory impairments (5, 6, 9, 14, 15, 18, 27, 29, 32, 33, 39, 40). Psychopharmacological studies have shown that reduction of cholinergic transmission by drugs such as scopolamine can produce impairments in young animals or humans that are similar in some ways to cognitive impairments seen in aged subjects (2–4, 18). However, attempts to improve cognitive performance in animals and humans by administration of cholinomimetic compounds such as physostigmine have been plagued by a relatively narrow range of effective doses, a large variability in response both between and within studies, and by a short duration of action (7, 13, 16, 26, 28, 30, 31, 34, 38).

The present study addressed these latter problems in at least two significant ways. First, we used a unique Go:No-Go repeated discrimination/reversal task to assess the effects of physostigmine on learning and memory. Secondly, we utilized chronic administration of physostigmine in a manner not studied in previous experiments with animals.

Traditionally, Go:No-Go reversal learning in animals involves the initial discrimination of two stimuli designated as S^+ and S^-. Responses in the presence of the S^+ are reinforced while responses in the presence of the S^- are not. When the subject is performing at a criterion level, the stimulus-response relationships are reversed. A baseline for gauging drug effects on learning and memory can be obtained by training animals until they acquire each successive reversal in a single session (10, 20, 23). Intrasession performance represents acquisition of that session's particular discrimination. Intersession performance tracks the acquisition and retention of general "solution strategies" (e.g., "win-stay/lose-shift") and also reflects proactive interference or transfer.

Experiment 1 examined the effects of chronic administration of physostigmine in a unique three-stimulus reversal paradigm. Rats were injected with physostigmine either pre- or postsession for 50 sessions (five sessions per week). Experiment 2 compared the acute effects of postsession physostigmine with each subject's own vehicle-injection reversal baseline, using the same subjects as in Experiment 1, in the more traditional two-stimulus successive reversal task.

EXPERIMENT 1

The purpose of Experiment 1 was to determine the effects of pre- and postsession chronic physostigmine on reversal acquisition during a series of three-stimulus discrimination problems. The behavioral test used in Experiment 1 was a three-stimulus reversal task involving one S^+ and two different S^- stimuli. The S^+ and one S^- were reversed each time criterion was achieved with one

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S^- remaining unchanged for the next problem. This procedure might provide two advantages over two-stimulus reversal problems. First, the addition of a second S^- might make the task more difficult than the two-stimulus reversal problem and thus more sensitive to drugs which enhance learning. Second, since no new learning is required for the nonreversed S^-, response probability in the presence of this S^- might reflect nonspecific effects of physostigmine on performance.

Improvement in performance following presession administration of physostigmine has been reported only with relatively low doses (21, 36, 38). In contrast, enhancement of performance in sessions following postsession injections of physostigmine requires much higher doses (1, 19, 31). Accordingly, rats were injected intraperitoneally either with 0.03 mg/kg physostigmine five minutes prior to each session (Group 1) or with 0.5 mg/kg physostigmine immediately after each session (Group 3). Groups 2 (presession) and 4 (postsession) were injected with vehicle. If physostigmine transitorily increases attention or stimulus discrimination or more directly affects processes underlying learning, the presession physostigmine group should solve more problems in 50 sessions than any other group. If physostigmine enhances memory processes, either positive or negative transfer effects could be observed; the effects of postsession physostigmine might depend on the number of discrimination sessions between reversal sessions. If the stimuli were constant over a number of sessions, physostigmine could increase the number of problems solved in 50 sessions through positive transfer within successive discrimination problems. On the other hand, negative transfer should occur between discrimination problems during reversal sessions. If reversals are frequent, this could decrease the number of problems solved in 50 sessions. Finally, pre- or postsession physostigmine may also affect acquisition of more general learning strategies, such as "win-stay" or the formation of reversal learning sets.

Performance controls are an important component of learning and memory experiments (22,35). Accordingly, rats were required on each trial to initiate the discriminative stimulus by responding on a separate observing response lever. This provided a general measure of responsivity and of motor or sensory impairment not specifically related to learning and memory. An additional measure of the same effects was provided by response probabilities to the nonreversed "performance control S^-"

METHOD

Animals

Twenty-four male Charles-River rats, 210–270 days old at the beginning of training, were housed two to a cage in a temperature-controlled room with a 12-hr/12-hr light-dark cycle. The rats were fed Purina Rat Chow ad lib, but were allowed free access to water for only 20 minutes following each session.

Apparatus

Operant chambers, $24 \times 25 \times 19$ cm, were enclosed in large, sound-attenuating cubicles. Two frosted glass levers requiring 25 g of pressure for switch closure were mounted on the front panel of each chamber 9 cm above the grid floor. Mounted above each lever was a bulb which could be illuminated by either a bright or dim light or by a bright light that flashed four times per second (0.125 second light on, 0.125 second light off). The bright light was approximately 20 times brighter than the dim. A drinking spout that projected 2.5 cm into the chamber between the levers on the front panel delivered 0.05 ml water. Programming and data collection were accomplished with a Texas Instruments 980A minicomputer.

Procedure

The rats were shaped to press the right lever in the presence of each discriminative stimulus (bright, dim or flash), then trained to press the illuminated left lever to initiate a discriminative stimulus. When the rats were initiating at least 100 discrimination response opportunities and responding to more than 80% of the discriminative stimuli within a session, they were advanced to the three-stimulus discrimination problem described below.

The left lever was illuminated for a maximum of 5 seconds, a response on this lever during this interval (an initiation response) produced a discrimination trial. If the rat did not respond, the lever was illuminated again after 5 seconds. A discriminative stimulus consisted of presentation of one of the three stimuli (bright, dim, or bright flashing light) for a maximum of 5 seconds arbitrarily designated S^+, S^-, and S^- for the first problem (balanced across rats). The stimuli were presented randomly on the right-hand bulb (above the response lever) with the restrictions that no one stimulus was presented more than four times consecutively, and that within each block of 40 trials, the S^+ was presented 20 times and the S^- stimuli were each presented 10 times. A lever press during the S^+ terminated the trial and produced reinforcement. A nonresponse to either S^- produced reinforcement after the trial. Lever presses during either S^- had no effect: the stimulus continued for the full 5 seconds. All errors (response failures to S^+, or responses to S^-) were followed by a correction trial with reinforcement available for a correct response. The intertrial interval was five seconds. Thus, each trial consisted of two parts: an initiation (observing) response opportunity and, following an initiation response, a discriminative stimulus response opportunity. Rats were trained on this task until they were responding during at least 50 observing response opportunities within a session. The next session was designated Session 1. Sessions were terminated one of four ways: after 90 minutes, after 300 initial trials, after 50 consecutive initiation response opportunities without a response, or when criterion was met. Criterion was defined as at least 90% correct responses to initial (noncorrection) trials for the most recent presentations of the three stimuli (18/20 responses to S^+ and 9/10 correct rejections for each of the two S^- stimuli). Following criterion performance, the stimulus-response contingencies were reversed for two of the stimuli the next session. Testing continued for 10 weeks, 5 sessions per week. Rats were divided into four treatment groups ($n = 6$ per group) based on performance during the last training session.

Drugs

Solutions of physostigmine (Sigma Chemical Co., St. Louis, MO) were prepared daily. Rats in the presession condition were injected intraperitoneally with physostigmine or distilled water vehicle in a volume of 1 ml/kg five minutes prior to each session. Subjects in the postsession condition were injected intraperitoneally within two minutes following session termination (a tone signalled the experimenter that a rat was finished).

Statistical Analysis

The total number of problems solved by each rat over 50 sessions were submitted to a two-way analysis of variance, and *t*-tests were used to test planned comparisons for significance. To determine group differences in S^+ or S^- response probabilities

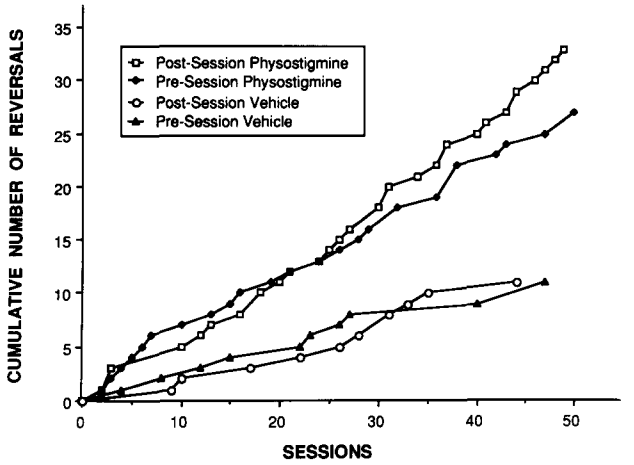

FIG. 1. Group comparison of the number of discrimination problems solved over 50 sessions, Experiment 1.

over time, Problem 1 was divided into three parts: Session 1, the Criterion Session, and a Midpoint Session (that session midway between Session 1 and the Criterion Session) for each subject. At each of these three points, response probabilities to S^+ and S^- initial (noncorrection) stimuli were calculated. These values were entered into a 4 (Groups) × 3 (Session) × 2 (Stimulus) analysis of variance with repeated measures on the last two factors. Post hoc analysis with the Tukey HSD test was used to elucidate the source of significant interactions.

The performance control measure was the number of initiation responses divided by the number of initiation response opportunities. This value was averaged over five consecutive sessions (a Block of sessions) to yield 10 probability scores for each rat. A 4 (Group) × 10 (Block) analysis of variance with repeated measures on the latter factor was used to determine group differences over time for the initiation response probabilities. To ascertain if the initial doses of physostigmine suppressed responding, initiation response probabilities during each of the first two sessions were individually examined with two-way analyses of variance.

RESULTS AND DISCUSSION

Physostigmine increased the number of problems solved in 50 sessions. The two-way ANOVA revealed a significant main effect of drug treatment, $F(1,20) = 12.45$, $p<0.05$, but no significant drug by injection time (pre- or postsession) interaction. Both presession physostigmine, $t(11) = 2.43$, $p<0.05$, and postsession physostigmine-treated rats, $t(11) = 2.59$, $p<0.05$, solved more reversal problems over 50 sessions than did comparably injected vehicle control groups. The mean number of problems solved over the 50 sessions by a subject in either of the two control groups (pre- or postsession) was 1.8. Rats in the presession physostigmine group solved a mean of 4.5 problems and subjects in the postsession physostigmine group solved a mean of 5.5 problems over 50 sessions. These data are illustrated in Fig. 1.

The $4 \times 3 \times 2$ repeated measures ANOVA revealed that all groups of subjects began Problem 1 by responding to the majority of both S^- and S^+ initial stimulus presentations. The probability of response to S^+ did not change significantly over sessions. All rats, regardless of treatment condition, achieved criterion by decreasing the probability of response to S^- stimuli over time. However, rats injected with physostigmine presession responded to significantly fewer S^- initial trials than did presession vehicle-injected subjects at both Session 1 (Tukey HSD test, $p<0.01$), and at the Midpoint Session (Tukey HSD test, $p<0.05$). Thus, a general reduction in nonreinforced responding was noted in subjects injected presession with a small dose of physostigmine. This replicates the findings of other investigators (8,12) who have reported similar reductions in S^- responding following administration of anticholinesterases.

The 4×10 repeated measures ANOVA carried out on the initiation response probabilities revealed no significant group differences or a group by block interaction. In the first block of five sessions, average response probabilities were 0.42, 0.48, 0.34, and 0.44 for the postsession physostigmine, postsession vehicle, presession physostigmine, and presession vehicle groups, respectively. Initiation response probabilities gradually increased for all groups over successive five-session blocks such that by the sixth block (asymptote), average response probabilities were 0.59, 0.59, 0.50, and 0.57, respectively. This resulted in a significant main effect of block, $F(9,27) = 13.75$, $p<0.001$. Although there was a trend for the presession physostigmine group to respond less frequently overall, this group was not significantly different from the other groups within any block. The same pattern was observed in the two-way ANOVAs for each of the first two sessions of the experiment: the presession physostigmine group tended to respond less frequently given an initiation response oportunity, but this difference was not statistically significant.

Since no subject achieved a daily reversal baseline, it was difficult to interpret response probabilities for the constant, performance control S^-. All rats, however, completed at least one problem. Therefore, response probabilities were compared for the performance control (nonreversed) S^- over two complete sessions: the Criterion Session of problem 1 and Session 1 of the first reversal (problem 2). There were no group differences in response probability at either session; probabilities increased from about 0.30 (for the entire Criterion Session) to chance (0.50) in all four treatments. Thus, at least for the first session of problem 2, it did not appear that any group was able to benefit from previously learned and constant stimulus-response relationships.

EXPERIMENT 2

Experiment 2 used as discriminative stimuli a bright light and a 2,500 Hz tone. In contrast to Experiment 1, where learning of each discrimination required many sessions, use of these more discriminable stimuli produced stable daily reversals. If physostigmine facilitated transfer between sessions, a postsession injection of physostigmine would be expected to interfere with performance on the reversed discrimination in the next session. This would increase the number of trials to criterion on sessions which followed physostigmine.

METHOD

Procedure

Stable baseline was defined as 10 consecutive sessions in which the subject's performance on daily reversals of the tone-light discrimination was greater than 70% correct responses over all initial (noncorrection) trials. In most cases, this level of performance also attained the criterion established for this experiment (24 correct out of the last 26 trials, 93.3%). All subjects used in Experiment 1 were tested for acquisition of stable baseline in this experiment. Seven subjects achieved baseline within 40 sessions, and were selected for drug challenge. Once this baseline was achieved, all sessions resulted in criterion performance.

Twice weekly, rats were injected intraperitoneally with either

0.5 mg/kg physostigmine (1 ml/kg) or with distilled water vehicle within two minutes of criterion performance. At least two sessions separated each injection, and vehicle injections alternated with physostigmine. Rats were injected six times with physostigmine postsession (three times after the light was S^+ and three times after tone was S^+) and six times with vehicle. The total number of trials to reach criterion in the next session was the dependent variable. The response probabilities over 20 consecutive trials (10 S^+ and 10 S^-) collected into "bins"; performance over consecutive 20-trial bins was used for a detailed analysis of within-session learning.

Statistical Analysis

Mean number of trials to criterion were obtained for each rat over its three treatment and three control sessions for each S^+ (light or tone). A 2 (Treatment) × 2 (S^+, tone or light) repeated measures analysis of variance was used to determine significant overall treatment differences.

In addition, response probabilities on S^+ and S^- initial stimuli were compared on control and treatment sessions for the first bin of 20 trials and for the bin in which criterion performance was attained under control conditions. The criterion bin was required to have at least 10 stimulus presentations. If criterion was reached before this occurred, those presentations were combined with the previous (complete) bin. Thus, a mean criterion bin number was determined under control conditions for each subject. Performance was recorded for S^+ and S^- initial stimuli during that same bin for each rat following physostigmine injections. A 2 (Treatment) × 2 (Bin, first or control-defined criterion) × 2 (Stimulus, S^+ or S^-) repeated measures ANOVA was used to compare response probabilities between conditions. Post hoc analysis was made with the Tukey HSD test. Initiation response probabilities were calculated for each subject and averaged over the six treatment and six control sessions. A repeated measures *t*-test compared response probabilities between physostigmine and vehicle treatments.

RESULTS AND DISCUSSION

The 2 × 2 ANOVA revealed that significantly more trials were required to reach criterion on days following postsession physostigmine injection (treatment session) than on days following vehicle injection (control session), $F(1,6) = 13.51$, $p<0.02$. Although the particular stimulus (e.g., light or tone as S^+) did not produce a significant main effect or interaction, the average points for light and tone are illustrated separately for each subject in Fig. 2.

As shown in Fig. 3, the 2 × 2 × 2 ANOVA revealed a significant treatment by stimulus (S^+ or S^-) interaction, $F(1,6) = 7.57$, $p<0.04$, and a significant treatment-by-bin-by-stimulus interaction, $F(1,6) = 9.10$, $p<0.03$. S^+ performance started and remained about 92% correct throughout the session following either physostigmine or vehicle; the increase in response probability was not significant in either group. During control sessions, rats rapidly discriminated S^+ and S^- trials. Thus, response probabilities for S^+ and S^- were significantly different in the first bin of 20 trials (Tukey HSD, $p<0.05$). In contrast, following physostigmine treatment, the difference between S^+ and S^- response probabilities did not achieve significance in the first bin.

At the criterion bin (defined by control performance), S^- response probabilities had significantly decreased from initial performance for both conditions. Rats in either treatment condition were clearly discriminating between the S^+ and S^- stimuli at this point. However, S^- response probabilities were significantly greater in sessions following physostigmine sessions than in sessions following vehicle sessions for this bin (Tukey HSD, $p<0.5$). S^+ response probabilities did not differ between conditions.

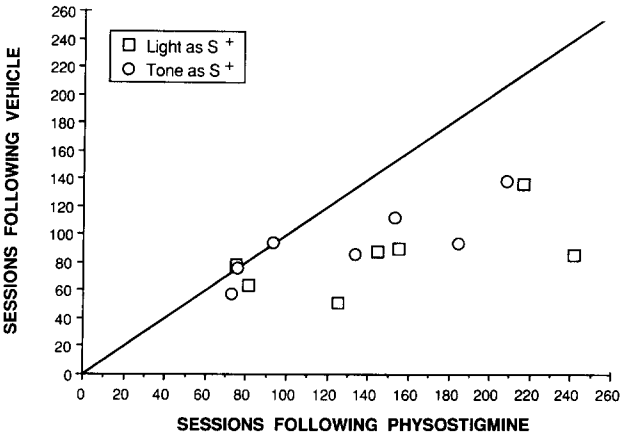

FIG. 2. Mean number of trials necessary to reach criterion for sessions following vehicle injection vs. sessions following physostigmine injection, Experiment 2. Each point is one subject's performance averaged over three control and three treatment sessions with either light or tone as S^+. Points below the line indicate that more trials were necessary to reach criterion for sessions after physostigmine injection than after vehicle injection.

Thus, postsession physostigmine impaired reversal learning in the next session. This negative transfer was selective for nonreinforced responding, and was evidenced both by poorer discrimination performance on the first 20 trials of the session and by retarded acquisition of nonresponding to the new S^-. As in Experiment 1, the initiation response probabilities were unchanged by physostigmine for any subject.

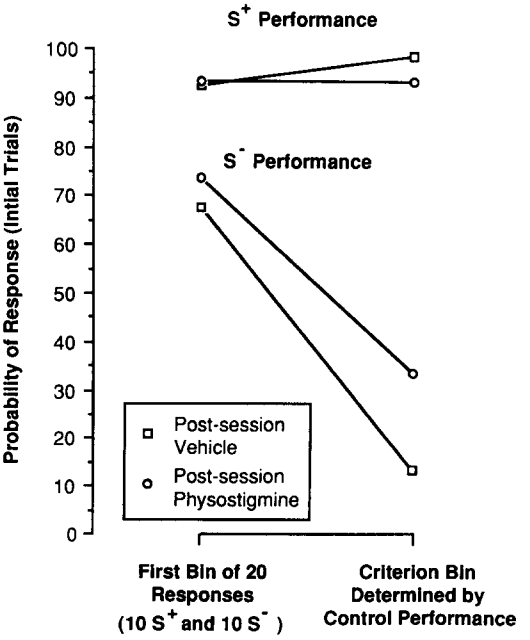

FIG. 3. Average response probabilities to discriminative stimuli for sessions following control injections over the first bin of 20 initial trials and over the criterion bin (see text). Response probabilities for the same 2 bins are illustrated for sessions following physostigmine injections.

GENERAL DISCUSSION

In Experiment 1, rats injected with physostigmine either presession or postsession learned significantly more problems over 50 sessions than did vehicle-injected control animals. Since postsession as well as presession administrations of physostigmine were effective, this facilitation of performance probably relates to some aspect of memory. Within the first discrimination, no differences were found in response bias or in patterns of responding, except that rats injected with physostigmine presession responded to fewer S^- stimuli over the first half of the first problem. Experiment 2 showed that in a daily reversal situation, postsession physostigmine impaired performance on the next session which again implicates memory processes. Further, this impairment was evidenced by a diminished withholding of responding to new S^- stimuli.

These results suggest that postsession physostigmine treatment enhanced memory of a previously learned discrimination, and is consistent with earlier reports (1, 19, 31). Thus, physostigmine improved acquisition through positive transfer within discrimination problems in Experiment 1, where the majority of sessions for any subject were not reversals. In the daily reversal paradigm of Experiment 2, where the discrimination learned on a session was incorrect on the next session, physostigmine retarded subsequent acquisition (negative transfer). The opposite effects of postsession physostigmine in Experiments 1 and 2 renders unlikely the possibility that physostigmine could have facilitated reversal learning set acquisition. Moreover, if postsession physostigmine could affect acquisition of learning set, it should have been without effect in Experiment 2, where injections were not begun until rats had attained a stable baseline of reversal acquisition.

That presession physostigmine and postsession physostigmine were equally effective in Experiment 1 may be due to residual effects of presession physostigmine which persisted after the session. Alternatively, physostigmine presession may have affected learning or nonspecific performance factors. Although we believe that the effectiveness of postsession physostigmine strongly implicates central processes, the fact that neostigmine was not included in the present studies means that we cannot fully reject the possibility that peripheral effects of either pre- or postsession physostigmine contributed to the results obtained.

Under control conditions, the rats in Experiment 2 responded on most of both S^+ and S^- trials during the first 20 initial trials of every reversal session. This suggests that the rats approached each new session as a new problem, or at least rapidly abandoned the previously learned discrimination rules. Similar results have been observed in Go:No-Go reveral procedures in pigeons (41). That responding on S^+ trials was initially high, whereas nonresponding on S^- trials was achieved only gradually, may also indicate a bias toward responding rather than nonresponding. Alternatively, the rat might have applied a "win-stay" strategy to each reversal problem, resulting in maintained high probability of response to the new S^+ and gradual extinction of responding to the new S^- stimulus.

Postsession physostigmine apparently did not alter any strategy or bias since the rats consistently responded on most S^+ and S^- trials at the beginning of the postdrug sessions in Experiment 2 as well. However, postsession physostigmine in Experiment 2 retarded learning of appropriate (non)responding on S^- trials of the subsequent (reversed) discrimination. The effects on S^- trial learning were presumably due to negative transfer of the opposing discrimination learned the previous session, prior to drug administration. The failure of postsession physostigmine to affect performance on S^+ trials may have been obscured by the rats' win-stay strategy.

Effects of physostigmine and other anticholinesterases are notoriously sensitive to dosage levels, and tend to vary between subjects. A great advantage of the method of Experiment 2 and of repeated acquisition procedures in general is that once daily reversal baseline has been achieved, an individual animal can be tested repeatedly with a number of different drug and dose combinations. Thus a drug could be "titrated" to determine optimal dosage, and different drugs could be systematically compared. The present results further suggest that a repeated acquisition procedure might be particularly useful in examining the role of the cholinergic system in learning and memory.

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