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THE ACQUISITION OF RESPONDING WITH CONDITIONED REINFORCEMENT: EFFECTS OF COCAINE, (+)-AMPHETAMINE AND PIPRADROL

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1 A procedure for examining the acquisition of a lever-pressing operant with conditioned reinforcement was used to compare the effects of three psychomotor stimulants.

2 Hungry rats were trained to associate an auditory tone (i.e., conditioned reinforcer) with food. Preference for the tone was then measured after treatment with pipradol (5, 10, 15 mg/kg), cocaine (1, 5, 10 mg/kg) or (+)-amphetamine (0.5, 1.5, 5.0 mg/kg).

3 In agreement with previous data, 10 mg/kg of pipradrol enhanced the effect of conditioned reinforcement whereas animals treated with any of the doses of (+)-amphetamine showed no effect.

4 Rats treated with cocaine (1 or 5 mg/kg) showed an effect of conditioned reinforcement but the effect was not significantly greater than in controls.

5 The present data suggest important differences in enhancement of responding for conditioned reinforcement by various drugs in the psychomotor stimulant class. These differences in turn may be related to the pharmacological actions of these compounds on release of catecholamines from different storage pools.

Introduction

Reinforcement or reward is usually defined as the contingent presentation of a stimulus (e.g., food) that increases the frequency of the response that preceded it. Stimuli that repeatedly are associated with reinforcement can acquire this ability to strengthen responses. However, these conditioned reinforcers usually are weaker than unconditioned reinforcers and they lose their ability to strengthen responses with repeated presentations in the absence of unconditioned reinforcement. It is now well established that certain psychomotor stimulant drugs can be used to enhance responding for conditioned reinforcement (Hill, 1970; Robbins, 1975; 1976; Robbins & Koob, 1978; Beninger, Hanson & Phillips, 1980). The initial study measured conditioned reinforcement during extinction of a previously trained lever pressing response. Pipradrol (10 mg/kg) greatly enhanced responding in extinction when presentation of a stimulus previously associated with unconditioned reinforcement was contingent upon the response (Hill, 1970).

Robbins (1975; 1978) extended Hill's original observation by employing sophisticated procedures that involved acquisition of a new response (i.e., transfer of control) and a choice paradigm (Mackintosh, 1974); he demonstrated that enhanced responding in extinction was maintained by the reinforcing properties of the conditioned reinforcer, rather than its discriminative properties. It should be noted that in a number of these studies, undrugged control animals, although showing a trend towards conditioned reinforcement, failed to show a significant effect (Robbins, 1975; 1978; Robbins & Koob, 1978). This indicates that conditioned reinforcement effects are often weak; in spite of this, pipradrol (10 mg/kg) reliably enhanced only responding producing stimuli previously associated with reinforcement.

Differences have been noted regarding the effect on conditioned reinforcement of various psychomotor stimulant drugs (Robbins, 1978). Specifically, methylphenidate (15 mg/kg) had a similar effect to pipradrol but was weaker. Surprisingly, neither (+)-amphetamine (0.5, 1.5 or 3.0 mg/kg) nor the antidepressant drug nomifensine (5, 10 or 15 mg/kg) had significant facilitatory effects on responding for conditioned reinforcement. It was suggested that these differences may be related to subtle differences in the pharmacological action of psychomotor stimulant drugs on catecholaminergic (CA) neurones (Robbins, 1978). It has been found that (+)-amphetamine enhances release of newly synthesized CAs whereas pipradrol, methylpheni-

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date and cocaine enhance release of CAs stored in a reserpine-sensitive pool (Scheel-Krüger, 1971).

As noted above, cocaine may have a pharmacological action on CA neurones that is more similar to pipradrol than (+)-amphetamine. Previous data suggest that there are qualitative differences in the effects on conditioned reinforcement of various drugs in the psychomotor stimulant class. To test this possibility further, the following experiments compared the effects of cocaine (1.0, 5.0 or 10.0 mg/kg), (+)-amphetamine (0.5, 1.5 or 5.0 mg/kg) and pipradrol (5, 10 or 15 mg/kg) on the acquisition of a new response for conditioned reinforcement. The specific experimental procedure differed somewhat from those employed by Robbins (1975; 1976; 1978) but has been used successfully to demonstrate a blockade of conditioned reinforcement by the dopamine receptor blocker, pimozide (Beninger & Phillips, 1980).

Methods

Sixty-six male albino rats of the Wistar strain were housed individually in a climatically controlled colony room kept on a 12 h light/dark cycle. The rats weighed from 225 to 315 g and were maintained at 80% of their *ad libitum* feeding weights throughout the experiment.

Apparatus

The experimental environments consisted of four similar Plexiglas chambers (30.0 × 21.5 × 46.5 cm high), each housed in a ventilated sound-attenuating box and illuminated by an overhead light. Two of the chambers had wax paper-covered wooden floors and two had grid floors. Each chamber was equipped with two removable levers (7.7 × 4.4 cm), one being located in the middle of each 21.5 cm end wall at a height of 4.0 cm. The force requirement for the levers was about 0.10 N. In the middle of one of the sides at a height of 1.5 cm was a feeder cup. A 2900 Hz tone generator (Sonalert) was mounted in the ceiling of each sound-attenuating box. Environmental contingencies and data collection were controlled by solid state switching and timing devices (BRS/LVE) for one chamber and by a Data General Nova 3 computer for the remaining three.

Procedure

A series of ten different groups was tested according to an experimental design with three distinct phases. The first group (the saline group, $n = 6$) was included to investigate the conditioned reinforcement effect produced in vehicle-treated animals using this experimental procedure. The following paragraphs present a detailed account of the procedure used to train the saline group. A description of the procedures used in training and testing the remaining drug groups will follow.

The three phases of the experiment were referred to as the pre-exposure, conditioning and test phases. The pre-exposure phase consisted of six 40 min sessions of exposure to the chamber with the two levers present. There was one session per day for three days, two days in the home cage, then the remaining three sessions on the next three days. During this phase, depressions of one of the levers (the tone lever) resulted in a 3 s tone presentation while depressions of the other lever (the no-tone lever) had no prearranged consequences. Previous studies (Beninger & Phillips, 1980) revealed that almost all rats showed a preference for the same side; therefore the tone lever was always placed on the nonpreferred side. The dependent variables were the number of responses on each of the two levers.

The conditioning phase consisted of four 60 min sessions. There was one session per day for the two days following the pre-exposure phase, then two days in the home cage followed by the remaining sessions on the next two days. During the conditioning phase the levers were absent from the chambers and Plexiglas plates covered the resulting apertures. During each session the 3 s tone was presented 80 times according to a random time 45 s schedule; i.e., the average intertone interval was 45 s. Each tone presentation during the first conditioning session terminated with the delivery of one 45 mg Noyes Precision Food Pellet and pellet delivery occurred only after a random 33% of the tone presentations in the next three sessions. This partial pairing procedure was employed because it has been demonstrated to produce more durable conditioned reinforcement (Zimmerman, 1959; 1963; Knott & Clayton, 1966).

The test phase consisted of one 40 min session which occurred on the next day. Each rat in the saline group was injected (i.p.) with physiological saline (1.0 ml/kg) 10 min before this session. The two levers were again present in the chambers and again one produced the 3 s tone. For any rats that had averaged more responses on the tone lever during the last three pre-exposure sessions the tone lever was moved to the nonpreferred side for the test session. Conditioned reinforcement was observed as a relative increase in the number of responses made on the tone lever during the test phase as compared to the pre-exposure phase.

The training of the nine remaining groups was similar to that of the saline group except that drugs were administered before the test session. Thus, three groups received an i.p. injection of pipradrol hydrochloride (Merrell Co.) dissolved in sterile dis-

tilled water 10 min before the test; the doses were 5, 10 and 15 mg/kg (16.5, 32.9 and 49.4 μmol/kg) and the group size was 6, 8 and 6, respectively. Three groups (all $n = 6$) received cocaine hydrochloride dissolved in physiological saline in doses of 1, 5 and 10 mg/kg (2.9, 14.7 and 29.4 μmol/kg) immediately before the test. The last three groups ($n = 6$, 8 and 8) received (+)-amphetamine sulphate dissolved in sterile distilled water in respective doses of 0.5, 1.5 and 5.0 mg/kg (1.4, 4.1 and 13.6 μmol/kg) 10 min before the test session.

Results

The purpose of the pre-exposure phase was to familiarize the rats with the experimental environment and to determine the rate of pressing on the tone and no-tone levers before conditioning. These initial lever-pressing rates (presses per session) were calculated by averaging the number of presses on each lever over the last three sessions of the pre-exposure phase. Lever-pressing rates for the test phase were simply the number of presses on each lever during the one test session. Thus the data consisted of two pairs of numbers for each rat.

The results for the saline group are shown in Figure 1a. Note that the data are presented as square roots; this was done to satisfy the requirements of the statistics used (see below). The data indicate that the increase in responding on the tone lever from pre-exposure to test was greater than the increase on the no-tone lever. This greater relative increase in responding indicated that the tone had become a conditioned reinforcer.

This description of the results was supported only by a marginal effect in the appropriate statistical test. Thus, a two-way analysis of variance with repeated measures on both variables, viz., levers and phases, was carried out on the square roots of the data; the square root transformation was used to reduce the differences in the variance associated with the two phases. In this analysis a significant lever by phase interaction would indicate that the difference in the number of responses made on the two levers in the test was different from that difference in the pre-exposure phase. The results revealed that the phase by lever interaction was marginally significant, $F(1,5) = 3.89$, $P = 0.106$. Thus nondrugged control animals showed a small change in preference for the tone lever, indicating that the tone had acquired weak conditioned reinforcement properties. The untransformed means may provide a clearer indication of this effect; the mean (± s.e.mean) number of responses on the tone and no-tone lever in the pre-exposure and test phases were, respectively, 9.8 (± 2.8), 23.2 (± 4.7), 24.5 (± 9.2) and 24.7 (± 4.7).

The results for the pipradrol groups also are shown in Figure 1. All groups showed an overall increase in responding on the two levers from pre-exposure to test. However, whereas the groups receiving 10 and 15 mg/kg doses appeared to change their preference to the tone lever, the 5 mg/kg group did not.

The square roots of the data for each group were subjected to a two-way analysis of variance. In agreement with the above description, the results revealed

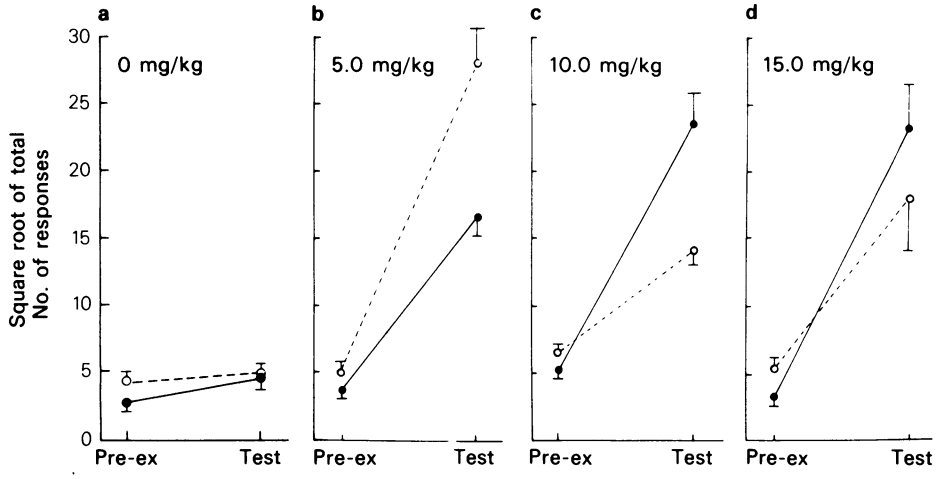

Figure 1 Mean square roots of number of responses on the tone (●) and no-tone (○) levers in the pre-exposure and test phases for the saline group and the three groups that received injections of pipradrol. Vertical lines show s.e.mean. All pipradrol groups showed a significant overall increase in responding (both levers combined). The relative increase for the tone lever was marginally greater than for the no-tone lever for the saline group. This increase was significant for the 10 mg/kg dose (c) only.

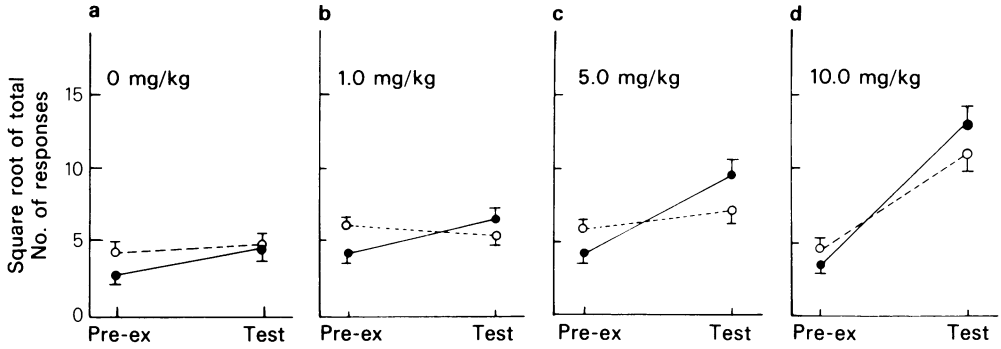

Figure 2 Mean square roots of number of responses on the tone (●) and no-tone (○) levers in the pre-exposure and test phases for the saline group (same as in Figure 1) and the groups injected with cocaine. Vertical lines show s.e.mean. The 5 and 10 mg/kg doses (c and d) produced a significant overall increase in responding (both levers combined). The relative increase in responding on the tone lever was significantly greater than for the no-tone lever for the two lower doses.

that all three pipradrol groups showed a significant overall increase in responding from pre-exposure to test, $F(1,5) = 158.41$, $P < 0.001$, $F(1,7) = 92.93$, $P < 0.001$, and $F(1,5) = 56.22$, $P < 0.001$ for the 5, 10 and 15 mg/kg doses, respectively. Only the group treated with 10 mg/kg of pipradrol showed a significant conditioned reinforcement effect; this was revealed by a significant two-way interaction of phases and levers, $F(1,7) = 22.73$, $P < 0.002$.

The results for the three doses of cocaine are shown in Figure 2. These data reveal that the groups receiving the two higher doses showed an overall increase in responding from pre-exposure to test. In addition, the three groups receiving different doses of cocaine appeared to show a change in preference for the tone lever from pre-exposure to test.

Statistical analysis of the square roots of the response totals for each of the cocaine groups revealed that the overall increase in responding was significant for the 5 and 10 mg/kg dose, $F(1,5) = 12.43$, $P < 0.02$ and $F(1,5) = 81.68$, $P < 0.001$, but not for the 1.0 mg/kg dose, $F(1,5) = 4.92$, $P > 0.05$. The analyses further indicated that the 1 and 5 mg/kg groups showed a significant phase by lever interaction, $F(1,5) = 9.69$, $P < 0.03$ and $F(1,5) = 11.02$, $P < 0.02$, revealing a significant conditioned reinforcement effect. This interaction was not significant for the 10 mg/kg dose, $F(1,5) = 2.46$, $P > 0.05$.

The results of the (+)-amphetamine groups are presented in Figure 3. The effects of this drug differed from the other two. Although each dose of (+)-amphetamine appeared to produce an increase in overall responding from pre-exposure to test, there was no significant conditioned reinforcement effect at any dose. From Figure 3, the 0.5 and 1.5 mg/kg doses appeared to produce a change in lever preference. However, for the 1.5 mg/kg dose this apparent effect was small and was caused by half of the animals in the group, the data being quite variable; for the 0.5 mg/kg dose, the magnitude of the

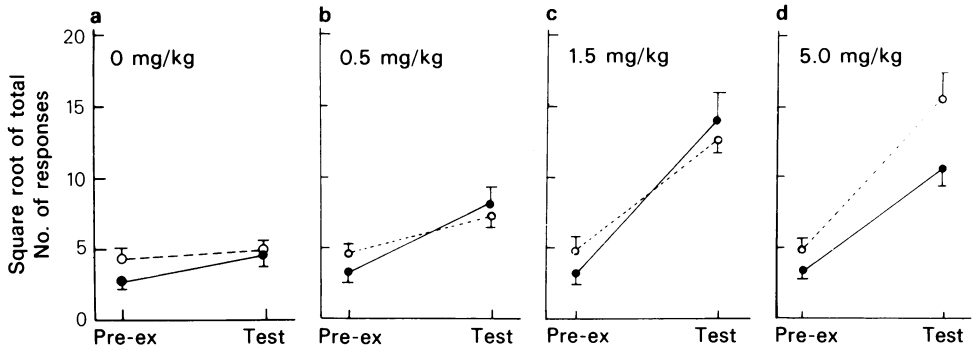

Figure 3 Mean square roots of number of responses on the tone (●) and no-tone (○) levers in the pre-exposure and test phases for the saline group (same as in Figures 1 and 2) and the groups that were injected with (+)-amphetamine. All groups showed a significant overall increase in responding (both levers combined) but none showed a significant change in lever preference from pre-exposure to test.

conditioned reinforcement effect was similar to the saline group.

These observations were confirmed by the results of individual two-way analyses of variance of the square roots of the response totals for each group. The tests revealed that the 0.5, 1.5 and 5.0 mg/kg doses of (+)-amphetamine produced a significant increase in responding from phase to phase, $F(1,5) = 107.7$, $P < 0.001$, $F(1,7) = 92.18$, $P < 0.001$, and $F(1,7) = 80.1$, $P < 0.001$, respectively. However, the phase by lever interaction was insignificant for each dose, $F(1,5) = 3.99$, $P > 0.05$, $F(1,7) = 1.56$, $P > 0.05$, and $F(1,7) = 3.00$, $P > 0.05$ for 0.5, 1.5 and 5.0 mg/kg doses, respectively, although the magnitude of this effect in the 0.5 mg/kg group was comparable to that in the saline group. Thus, although animals injected with (+)-amphetamine responded more in the test phase, they failed to show any significant evidence of conditioned reinforcement.

One final series of analyses was carried out to compare the magnitude of the conditioned reinforcement effect in the drugged groups to the control group. This was done by using three-way analyses of variance with repeated measures on two variables. The variables analysed were groups, levers and phases, the latter two being those with repeated measures. If one of these analyses yielded a significant three-way interaction, it would indicate that the two-way interaction of levers and phases (which is taken as evidence of conditioned reinforcement) differed for the two groups. These analyses compared each drug group that showed a significant conditioned reinforcement effect to the saline group. The results revealed that only the group receiving 10.0 mg/kg of pipradrol showed significant enhancement of the conditioned reinforcement effect, $F(1,12) = 11.92$, $P < 0.005$.

Discussion

In the present study, the saline group showed a trend towards conditioned reinforcement which failed to reach statistical significance ($P < 0.05$). This observation is entirely consistent with the findings from a number of previous studies (Robbins, 1975; 1978; Robbins & Koob, 1978), as is the observation of a significant conditioned reinforcement effect in some drug-treated groups. Thus, the effects of various psychomotor stimulants can be summarized as follows: pipradrol enhanced the effects of conditioned reinforcement only at one dose (10 mg/kg); cocaine treatment produced an effect of conditioned reinforcement at the two lower doses (1 and 5 mg/kg); (+)-amphetamine, on the other hand, failed to produce any indication that the tone had acquired significant reinforcing properties.

Treatment with pipradrol has failed to show a consistent relationship between dose and magnitude of conditioned reinforcement. There is general agreement that the 10 mg/kg dose enhances responding for conditioned reinforcement (Hill, 1970; Robbins, 1975; 1976; 1978; Robbins & Koob, 1978; Beninger *et al.*, 1980). However, both significant and insignificant effects have been reported with 5.0 and 15.0 mg/kg doses (Robbins, 1978; Robbins & Koob, 1978). Robbins & Koob (1978) suggested that differences in training procedures, primary reinforcers or age of the rats may account for these discrepancies.

Animals injected with the two lower doses of cocaine showed an effect of conditioned reinforcement as well as an elevation of general activity at the two higher doses. This former effect has not been reported previously. (+)-Amphetamine, on the other hand, produced a significant increase in activity but no significant effect of conditioned reinforcement was observed at any dose. This effect is in complete agreement with Robbins' (1978) observation of little responding on the conditioned reinforcement lever in animals treated with (+)-amphetamine.

In the present study, psychomotor stimulants produced a dose-dependent increase in total number of presses on both levers. However, most previous studies reported increases only on the lever producing conditioned reinforcement (e.g., Robbins, 1975; 1976; 1978) or no effect. This difference probably can be attributed to the size of the levers; the levers in the present study protruded 4.5 cm into the chamber and were much larger in surface area than those used by Robbins. This elevation in responding was independent of the conditioned reinforcement effect and can be viewed as an index of changes in general activity level. As such, the observation of increased activity with psychomotor stimulants replicates many previous reports (see Costa & Garattini, 1970). Yet, in spite of the common effect of pipradrol, cocaine and (+)-amphetamine on activity in general, these compounds were seen to have different effects on conditioned reinforcement.

In considering the different effects of pipradrol and (+)-amphetamine on conditioned reinforcement, Robbins (1978) speculated that the different pharmacological action of the two drugs might be relevant. Whereas pipradrol enhances the release of CAs from a reserpine-sensitive storage pool, (+)-amphetamine seems to enhance the release of newly synthesized CAs (Scheel-Krüger, 1971). Insofar as rats injected with cocaine showed conditioned reinforcement, the behavioural effects of cocaine were more like those of pipradrol than (+)-amphetamine. It is interesting to note, therefore, that cocaine also produces some of its neuropharmacological effects

by enhancing the release of CAs from a reserpine-sensitive storage pool (see Scheel-Krüger, 1971). In spite of this similarity in neuropharmacological action, other differences must exist as cocaine did not produce a significant facilitation of responding for a conditioned reinforcer. In this regard, cocaine is similar to methylphenidate which enhances the acquisition of responding for conditioned reinforcement but is less effective than pipradrol (Robbins, 1978) and which enhances the release of CAs from a reserpine-sensitive storage pool (Scheel-Krüger, 1971). Further study with these compounds that have different pharmacological actions may reveal the mechanisms underlying behavioural changes produced by conditioned reinforcers.

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