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The effects of (+)-amphetamine and apomorphine on responding for a conditioned reinforcer

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Abstract. Two psychomotor stimulants, (+)-amphetamine and apomorphine, were examined for effects on associative learning and responding for a conditioned reinforcer. The experimental phases included: 1) preexposure to an operant test chamber with two levers, each of which produced a neutral stimulus when pressed; 2) pairings of one stimulus with food; and 3) a subsequent test of lever pressing for the two stimuli. Groups of food deprived rats ($n=8$–12) were given IP injections of one stimulant prior to each pairing or testing session. Given during pairings, (+)-amphetamine produced a dose-related attenuation of responding for the conditioned stimulus in the test; doses of 0.5, 1.0 and 2.0, but not 0.25 or 4.0 mg/kg, given during the test enhanced responding, as did 0.5 mg/kg given in both phases. Apomorphine did not significantly alter responding during testing when administered in either the pairing (0–0.75 mg/kg) or test (0.5–1.0 mg/kg) phase. The results suggest that the modulation of conditioned reinforcement by psychomotor stimulants may occur through a presynaptic influence. Furthermore, the results with (+)-amphetamine suggest that this drug differentially affects the learning of an association between a conditioned and unconditioned stimulus versus the acquisition of responding for that conditioned stimulus.

Key words: (+)-Amphetamine – Apomorphine – Conditioned reinforcement – Dopamine – Rats

The behavioural effects of psychomotor stimulants have generated a great deal of research, possibly due to their widespread abuse. One aspect that has encouraged numerous studies is their enhancement of operant responding for primary reinforcing stimuli (for reviews, see Lyon and Robbins 1975; Sanger and Blackman 1976). These results have also been extended to include responding for conditioned reinforcers, neutral stimuli that acquire properties through association with a primary reinforcer (Hill 1970).

With the utilization of a conditioned reinforcement (CRt) paradigm, it has been demonstrated that pipradol leads to an increase in responding for a conditioned reinforcer, rather than simply increasing non-specific responding on a second lever that does not produce that stimulus (Beninger et al. 1980, 1981; Robbins 1978; Robbins et al. 1983). Similar results have been observed with certain cocaine analogues and (+)-amphetamine (Robbins et al. 1983). Surprisingly, apomorphine, although increasing responding in general, failed to produce a selective enhancement for a conditioned reinforcer in the one study published so far (Robbins et al. 1983). These findings may be related to the different ways in which the stimulants act; apomorphine directly stimulates dopaminergic receptors whereas pipradrol, (+)-amphetamine, and cocaine analogues enhance neurogenic release of the catecholamines (Colpaert et al. 1976; Robbins et al. 1983; Scheel-Kruger 1971; Westerink 1979).

The enhancement of CRt observed with amphetamine and similarly acting drugs has been ascribed to at least two factors. Hill (1970) suggested that the drugs increase the rewarding quality, or response eliciting capabilities, of stimuli. Lyon and Robbins (1975) postulated that the effects could be attributed to the stimulatory action of the drugs, greater increases being observed with responses that initially had higher probabilities of occurrence. As dopamine is thought to have a role in modulating both reinforcement and activity (Beninger 1983), it seems likely that both processes may be influencing the observed changes in responding.

The extent to which the reinforcing and the motoric effects are each involved is difficult to assess. However, by testing animals drug free for CRt, any immediate motoric influences would be eliminated. As more salient reinforcers are known to elicit more responding (Keesey and Kling 1961; Shettleworth and Nevin 1965), it might be expected that drug administration during the pairing phase would increase responding for the conditioned reinforcer during the testing phase if it is affecting reward.

The present experiment was undertaken to further examine the interactions of (+)-amphetamine and apomorphine with responding for a conditioned reinforcer. A paradigm was used that has been shown to reliably produce CRt with paired stimuli, little effect with the primary reinforcer alone, and no effect with the stimuli negatively correlated (Hoffman and Beninger 1985). For further analysis of the paradigm, the reader is referred to the original article. Drugs were administered during either the pairing or the testing phase in an attempt to further delineate the contribution of any possible reward enhancing properties from the effects of general motor excitation on lever pressing for a conditioned reinforcer.

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Materials and methods

Subjects. A total of 154 male albino rats of the Wistar strain (Charles River, Canada) were individually housed in a climatically controlled ($21 \pm 1°$ C) colony room kept on a 12-h light (0600–1800 hours)/dark cycle. They were fed a restricted diet of Purina Rat Chow to maintain them at 80% of free feeding weights of 250–300 g. Water was freely available.

Apparatus. Four similar chambers ($23.0 \times 29.0 \times 17.5$ cm) were each ventilated by a small fan and enclosed in a Styrofoam insulated plywood box. The chambers had two 2 W lights mounted 6 cm from the ceiling situated 4.2 cm from the center of the back wall. A 4.9 Khz (Sonalert) sound generator was located between the lights. The tones (87–95 db) were set at 10 db above background noise levels. A removable lever (4.7×7.5 cm) was placed 2 cm from the floor in the center of each of the side walls. A food cup, connected to a Colbourne (G5100) 45 mg pellet dispenser, was mounted 2.5 cm from the floor below the sound generator. Each cup had an infrared emitter and detector that determined when the rat's snout entered it. An LSI/11/2 (Digital) microcomputer controlled contingencies and recorded data.

Procedure. The rats were divided into groups ranging in size from 8 to 12. All were treated identically, with the exception of drug treatment as described below. For each group, the 11-day study was divided into three distinct phases, termed preexposure, conditioning and test.

The preexposure phase consisted of five 40-min sessions where the number of responses on each lever was recorded for each rat. Only the last 30 min from each session was tabulated as these data provided the most stable estimate of baseline rates (Hoffman and Beninger 1985). One lever produced a 3-s tone, the other turned off the lights for 3 s. The position of the "tone" and "light-off" levers was counterbalanced across chambers.

The conditioning phase consisted of four 60-min sessions. The levers were removed from the chambers and the rats received pairings of the light-off stimulus with 45 mg food pellets (Bioserv). Only the light-off stimulus was paired with food as preliminary studies had shown that the tone paired with food did not produce CRt. In each session 80 3-s light-off presentations were given on a 45-s random time schedule. In the first session food followed each stimulus, but in the last three, food was presented only on a random 33% of the trials. Such partial pairing techniques have been shown to produce more reliable CRt effects (Knott and Clayton 1966). If any rat failed to eat the pellets, his data were discarded from subsequent analyses.

The test phase consisted of two 40-min sessions. Conditions were identical to those in preexposure. Again the number of responses on each lever was recorded during the last 30 min of each session.

Drugs and drug groups. (+)-Amphetamine sulphate (Smith, Kline and French) was dissolved in distilled water. Apomorphine hydrochloride (McFarlan-Smith) was also dissolved in distilled water with the addition of 1 mg of ascorbic acid per ml of water. Both drugs were injected intraperitoneally (IP) at a volume of 1 ml/kg body weight. All injections were administered within 5 min of the beginning of a session.

Four groups of rats (all $n=8$) received (+)-amphetamine in doses of 0 (0.9% saline), 0.13, 0.25, or 0.5 mg/kg prior to each conditioning session. Five groups (all $n=8$) received doses of 0.25, 0.5, 1.0, 2.0, or 4.0 mg/kg prior to each test session. One group ($n=10$) received 0.5 mg/kg prior to each conditioning and test session.

Five groups of rats received apomorphine in doses of 0 (ascorbic acid), 0.06, 0.13 (all $n=8$), 0.5, or 0.75 (both $n=12$) mg/kg prior to each conditioning session. Three groups (all $n=8$) received doses of 0.5, 0.75, or 1.0 mg/kg prior to each test session.

Results

The mean number of responses on each lever during the test phase is shown for all groups in Fig. 1. Data from rats given the same drug in the same phase were analysed in a three-way analysis of variance with phase, lever and group as the factors. The three-way interaction was significant for groups given (+)-amphetamine during conditioning ($F=5.49$, $df=3,25$, $P<0.005$), and during testing ($F=7.35$, $df=5,40$, $P<0.001$). However, the interactions were not significant for rats given apomorphine during conditioning ($F=0.50$, $df=4,34$, n.s.), or during testing ($F=1.97$, $df=3,22$, n.s.).

Response rates for the two stimuli during preexposure were found to be consistently low across groups, ranging from 0.69 ± 0.12 to 8.60 ± 2.70. However, the group receiving 0.06 mg/kg apomorphine during conditioning showed an aberrant rate (18.32 ± 4.57) on the light-off lever which

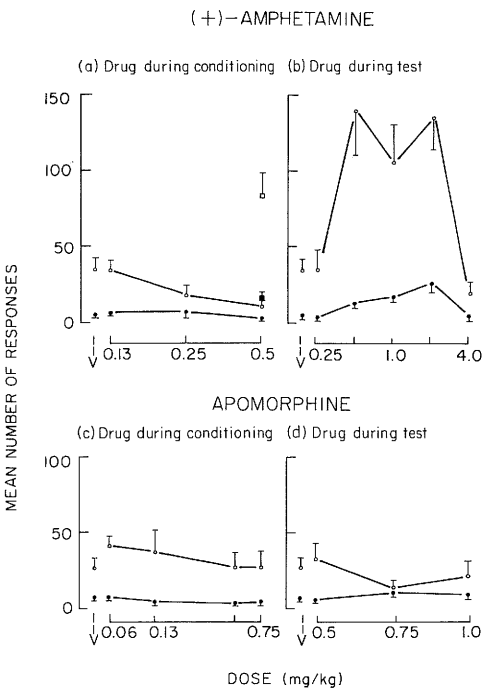

Fig. 1 a–d. Mean (±SEM) number of responses on the light-off (upper trace) and tone (lower trace) levers in the test phase for groups given (+)-amphetamine in conditioning (**a**), in the test (**b**), apomorphine in conditioning (**c**) or in the test phase (**d**). Vehicle (*V*) groups in (**b**) and (**d**) received injections during conditioning. The additional group shown in (**a**) (*square symbols*) was given the drug in the conditioning and test phases

was assumed to be due solely to random variation. Thus, the significant interactions observed with (+)-amphetamine suggest that responding for the light-off stimulus during the test phase accounted for the effects as preexposure rates on both levers, and test phase rates on the tone lever were relatively stable. In particular, there appeared to be a dose-related decrease in responding for the light-off in the test when (+)-amphetamine was administered in conditioning; rats given the same drug in the test phase appeared to show an inverted U-shaped function, with responding for the light-off stimulus being maximally enhanced with the mid-dose range. To determine if these effects were reliable, analyses of trend were conducted on these data for both sets of rats given (+)-amphetamine. There was a significant linear trend with the drug in conditioning ($F=10.56$, $df=1,25$, $P<0.005$), and a significant quadratic trend with the drug in the test phase ($F=25.81$, $df=1,40$, $P<0.001$), suggesting that there were indeed dose-related changes in responding for the light-off stimulus in the test phase.

The finding that there was a trend towards less responding for the light-off in the test phase when (+)-amphetamine was administered during conditioning may possibly be explained by alterations in stimulus pairings due to some non-specific drug effect. Thus, the latencies to feed during the four conditioning sessions were compared for these four groups. The number of occasions where the rats ate within 2 s of the total (152) food presentations were analysed in a between-subjects analysis of variance. However, the results were not significant ($F=0.90$, $df=3,25$, n.s.). The mean frequencies (±SEM's) for saline, 0.13, 0.25 and 0.5 mg/kg were 127.17 (±4.05), 117.75 (±7.82), 109.5 (±8.49) and 114.57 (±7.45), respectively.

To determine if there were differences in the CRt effect between individual groups and their control, each pair was examined in a three-way analysis of variance with phase, lever, and groups as the factors. The three-way interaction suggests that the phase by lever interaction differs between the two groups. All (+)-amphetamine groups, except those given the lowest dose during conditioning, and those given the lowest or highest dose during the test phase, showed significant interactions, whereas there were no significant effects with apomorphine.

To examine CRt further, the phase by lever interaction was examined for each group alone, as shown in Table 1. Of the groups given (+)-amphetamine, all showed significant interactions except those given 4.0 mg/kg in the test phase and those given 0.5 mg/kg in conditioning. Post hoc tests determined that of the groups showing interactions, all had significant increases in responding on the light-off lever from the preexposure to test phase. The group given 0.5 mg/kg in conditioning and test, and those given 0.5, 1.0, and 2.0 mg/kg in the test phase alone, also showed increases in responding on the tone lever across phases. However, the finding that there were interactions, and that responding was greater on the light-off lever, indicates a differential enhancement of responding for that stimulus as opposed to the tone. With apomorphine, all groups except those given the two higher doses during the test phase exhibited significant phase by lever interactions. Again, post hoc tests determined that in most of these cases was an increase across phases in responding on the light-off lever but not the tone. The control group however, also had a significant increase in responding on the tone lever. Rats treated with 0.5 mg/kg in the test phase, although showing an interaction, failed to exhibit significant results with post hoc tests on either lever.

Table 1. *F*-ratios and significance levels for the phase x lever interaction for each group and post hoc tests of phase effect for each lever

Phase	Group	*n*	*F*	*P*	Post hoc phase effect	
					Light-off	Tone
(+)-Amphetamine						
Cond	veh	8/6	17.06	<0.01	<0.05	n.s.
	0.13	8	16.73	<0.01	<0.05	n.s.
	0.25	8	7.12	<0.05	<0.05	n.s.
	0.5	8/7	5.43	0.05<*P*<0.06	<0.05	n.s.
Test	0.25	8	6.96	<0.05	<0.05	n.s.
	0.5	8	19.07	<0.005	<0.05	<0.05
	1.0	8	20.12	<0.005	<0.05	<0.05
	2.0	8	29.92	<0.005	<0.05	<0.05
	4.0	8	2.59	n.s.		
c/t	0.5	10/8	23.53	<0.005	<0.05	<0.05
Apomorphine						
Cond	veh	8	5.72	<0.05	<0.05	<0.05
	0.06	8	12.61	<0.01	<0.05	n.s.
	0.13	8	7.81	<0.05	<0.05	n.s.
	0.5	12/9	7.11	<0.05	<0.05	n.s.
	0.75	12/6	7.94	<0.05	<0.05	n.s.
Test	0.5	8/5	9.74	<0.05	n.s.	n.s.
	0.75	8/7	0.13	n.s.		
	1.0	8/6	1.69	n.s.		

Original sample sizes, and the number used in the analyses are shown

Returning to the three-way interactions with individual groups compared to the vehicle, the data suggest a difference in the extent to which a group showed CRt in comparison to the control, as in all cases with three-way interactions both the vehicle and the drug group showed CRt. These data, taken in conjunction with the results from the trend analyses suggest that (+)-amphetamine in conditioning attenuated responding for the conditioned reinforcer whereas with the same drug in the test phase responding was enhanced.

Discussion

The present study determined that (+)-amphetamine influenced responding for a conditioned reinforcer whether the drug was administered in either the pairing or the testing phase. However, these effects were quite dissimilar; given during conditioning there was a dose-dependent attenuation, whereas given in the test phase the mid-dose range enhanced responding for the conditioned stimulus. It is interesting to note the dissimilar results with 0.5 mg/kg, depending on whether the drug was given during the conditioning or the test phase. Furthermore, rats given this dose in both phases more closely resembled those having the drug in the test than in conditioning.

Previous investigations of (+)-amphetamine and responding for conditioned reinforcers (with the drug given

in the test phase) have yielded mixed results. In one case a general increase in lever pressing was observed (Beninger et al. 1981); another found no increase in pressing for the paired stimulus (Robbins 1978) and yet another showed a selective increase in responding for the conditioned reinforcer (Robins et al. 1983). The present results appear similar to those of Robbins et al. (1983), although significant effects were found here with much smaller sample sizes.

The dose-dependent decrease in responding for the light-off stimulus when (+)-amphetamine was given in the conditioning phase raises the possibility of state-dependent drug effects (Overton 1974). The group given the drug in both phases exhibited a stronger effect than the group given the same dose in conditioning alone, a finding that would suggest the presence of state dependency. However, groups given the drug in just the test phase showed similar effects. If state dependency were a major factor here, it might be expected to similarly affect both sets of groups that received drugs in just one of the two phases.

It is possible that there were motivational differences between rats treated with (+)-amphetamine or the vehicle during conditioning, particularly as this drug has demonstrated anorectic properties (Kornblith and Hoebel 1976). However, it was noted that there was no significant group effect in the frequency of feeding within 2 s of food delivery for rats receiving various doses of (+)-amphetamine during the pairing phase. Although this finding does not preclude motivational variables, it suggests that they were not responsible for the observed decrease in magnitude of the CRt effect.

The results with apomorphine appeared to be in direct contrast to those of (+)-amphetamine. Groups receiving doses of 0.75 and 1.0 mg/kg in the test phase failed to show significant phase by lever interactions, suggesting that higher doses of apomorphine may actually impair the acquisition of responding with CRt. However, in neither the conditioning nor the test phase did the drug significantly alter the CRt effect from that seen in the control group. Indeed, there was little variability in responding on either lever with any dose tested.

Robbins et al. (1983) similarly found that apomorphine did not differentially increase responding for a conditioned reinforcer, but rather increased response rates in general. Furthermore, each rat, while under the influence of the drug, appeared to prefer one lever, regardless of whether or not it produced the conditioned reinforcer. The present study did not find this same result. However, Robbins et al. (1983) utilized a wider dose range, and injected the drug subcutaneously. It has been suggested that subcutaneous injections are approximately five times as potent as IP injections with this drug (Ungerstedt et al. 1977). These variables may have contributed to the differences in results.

Thus it appears that (+)-amphetamine and apomorphine have differential effects on responding for a conditioned reinforcer. Furthermore, the way in which (+)-amphetamine affects responding depends on whether the drug is administered during the learning of the association or during the testing after learning has already occurred. Both of these drugs are known to enhance transmission within dopaminergic systems. However, they do so by different means. Apomorphine acts by directly stimulating dopaminergic receptors (Colpaert et al. 1976; Westerink 1979). (+)-Amphetamine, on the other hand, enhances the neurogenic release of dopamine and blocks reuptake, thus prolonging its action at receptor sites (Scheel-Kruger 1971). Although (+)-amphetamine interacts with transmitter systems other than dopamine, the behavioural effects of this drug appear to be primarily dopaminergic in nature (Moore 1977). The results of the present study suggest that significant modifications of CRt apparently are dependent on the alteration of ongoing neuronal activity, rather than simply stimulation of dopaminergic receptors.

The findings with (+)-amphetamine do not preclude the possibility that the effects of this stimulant interact with the rewarding quality of stimuli. When given during testing the results may be interpreted in relation to either motor or reward effects. However, when given during conditioning any immediate motoric effects should not influence bar pressing in the test phase. The (+)-amphetamine results may be related to the drug's ability to influence the rewarding capacity of stimuli; it is possible that for rats drugged during conditioning, not only did the stimulus (light-off) explicitly paired with food become a conditioned reinforcer, but also other environmental stimuli acquired similar properties. Possibly, as a result, the effectiveness of the light-off stimulus in controlling responding in the test was reduced. Such an interpretation might account for the attenuation of CRt observed with (+)-amphetamine when administered during learning.

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