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Discriminative Stimulus Properties of *d*-Amphetamine in Pigeons

TORBJÖRN U. C. JÄRBE

University of Uppsala, Department of Psychology, P.O. Box 227, S-751 04 Uppsala, Sweden

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JÄRBE, T. U. C. *Discriminative stimulus properties of* d-*amphetamine in pigeons.* PHARMAC. BIOCHEM. BEHAV. 17(4) 671–675, 1982.—Two out of four pigeons were successfully trained in an operant procedure to discriminate between the presence and absence of the effects induced by *d*-amphetamine (final dose: 1.6 mg/kg). The solvent (saline) or *d*-amphetamine was administered intramuscularly (IM) 30 min prior to training. Tests with other drugs and dosages indicated that *l*-amphetamine (ED_{50}=0.55 mg/kg) and cocaine (ED_{50}=1.05 mg/kg) fully generalized to *d*-amphetamine (ED_{50}=0.35 mg/kg), whereas drugs such as *p*-hydroxy-amphetamine (1.6 and 3.2 mg/kg), morphine (1.5, 3.0 and, 6.0 mg/kg), and Δ^9-THC (0.125, 0.25, and 0.50 mg/kg) failed to do so at the doses tested. Apomorphine (0.25 and 0.50 mg/kg) and LSD-25 (0.04 and 0.08 mg/kg) produced intermediate results. Pretreatment with haloperidol (dose range: 0.04 to 1.28 mg/kg), but not propranolol (10 and 20 mg/kg), attenuated significantly the *d*-amphetamine (1.6 mg/kg) stimulus effects. The two pigeons emitted predominantly *d*-amphetamine appropriate responses when the training dose (1.6 mg/kg) of *d*-amphetamine was tested on different occasions 15, 60, and 120 min after the administrations. One bird emitted mostly vehicle appropriate responses when tested 240 min after the *d*-amphetamine injection whereas the other bird performed *d*-amphetamine appropriate responses. Selection of the non-drug associated key occurred in the two birds when testing was carried out 480 min (8 hrs) after the administration of *d*-amphetamine.

d-Amphetamine stimulus Generalization Antagonism Pigeons

DRUG effects can be used to control the emission of a differential response pattern in animals in much the same way as external stimuli (light, sound, etc.) are used to control the behavior of organisms. That is, drugs are established as discriminative stimuli (DS) implying that the presence and absence of the drug effects indicate which of the two alternative responses will be reinforced during a particular training session. The most frequently used species in drug discrimination research has been the rat, thus available information concerning discriminable properties of drugs in other species is sparase. Species comparisons provide insight into pharmacologic organization and variation at different evolutionary levels. The comparatively long life-span of pigeons is of advantage since animals can be used repetitively once they have been trained on a given drug-discrimination task. The present study provides information on some discriminable effects of *d*-amphetamine in pigeons, a drug stimulus widely studied in rats [11]. Tests included other central nervous system (CNS) stimulant drugs, apomorphine, morphine, Δ^9-THC and LSD to determine commonality and specificity of the *d*-amphetamine stimulus in pigeons. Tests for antagonism included haloperidol and propranolol.

METHOD

Animals

The subjects were 4 experimentally naive, mature male pigeons of a mixed strain (Estuna AB, Sweden). Between experimental sessions the birds were individually housed in a larger colony room (lights from 8:00–20:00 hr; temperature 20°–22°C; humidity 50%–60%). During the experiments they were maintained at about 80% of their respective free-feeding weights through food deprivation. Water and oyster shell grits were freely available in the home cages.

Apparatus

The experimental chamber was identical to that previously described [15]. The response keys, 2 cm in diamter and dimly illuminated with white light, were mounted horizontally 10 cm apart on the front panel of the chamber, each key about 19 cm above the chamber floor. The opening of the key contact defined the key-pecking response. The minimum force necessary to operate a key was about 15 g. The food magazine was located between the response keys, 4 cm above the floor of the chamber. Conventional relay programming and recording equipment, located in a room adjacent to that of the chamber, were used. White noise was present in the chamber at all times and the chamber was ventilated by an exhaust fan.

Procedure

Discrimination learning. Initially the birds were shaped to obtain food by pecking on the right hand key, the left key being covered with tape. Once this requirement was met, *d*-amphetamine was given prior to the sessions and the birds had to peck the left key to get food; the right hand key now was covered with tape. Such "forced" training with inappropriate key covered was continued for 20 sessions and the requirement for obtaining grain was increased until the birds pecked the key 15 times (FR 15); the FR 15 schedule was in operation for all birds after 6 sessions. Half the number of

sessions were preceded by saline and the other half by *d*-amphetamine.

During the free-choice discrimination situation both keys were available and the birds had to respond on the appropriate key to produce food. Which key was appropriate depended on whether *d*-amphetamine or saline had been given. Responses on the inappropriate key were recorded but had no programmed consequences. Discrimination training followed a single alternation design (*d*-amphetamine, saline, *d*-amphetamine, saline, etc.) and the birds were trained three times per week (Mondays, Wednesdays and Fridays), 15 min per session. The drug training condition (D) consisted of an intramuscular injection of *d*-amphetamine, initially 1 mg/kg and later increased up to 1.6 mg/kg, and the non-drug condition (N) was 1 ml/kg of saline. The solutions were given 30 min prior to the session. The birds were trained during 64 sessions with the dose of 1 mg/kg vs saline, 30 sessions with a dose of 1.4 mg/kg vs saline, and 20 sessions with 1.6 mg/kg of *d*-amphetamine vs saline before testing. At this point two pigeons (P 27 and P 29) were switched from the training procedure to the test procedure; these birds had performed 100% correct *d*-amphetamine (1.6 mg/kg) choices and 100% and 90% correct saline choices respectively during the last 20 sessions prior to the first test session. The sequence for training under *d*-amphetamine or saline on Mondays and Wednesdays and testing (T) on Fridays became, N, D, T (Week 1), D, N, T (Week 2), N, D, T (Week 3), etc. During testing, the two pigeons could obtain 15 rewards if all responses (225 pecking responses) were directed to the key on which the bird first completed 15 responses (selected key). Once one key was selected, pecking on the other, non-selected key, did not activate the food magazine. Test sessions were preceded by at least one *d*-amphetamine and one saline training session. Tests were not conducted unless the performance during the preceding training sessions had been on the correct manipulandum.

Drugs. *d*-Amphetamine SO_4, *l*-amphetamine SO_4, cocaine HCl, morphine HCl (ACO, Sweden), *para*-hydroxyamphetamine HBr (Smith, Kline and French, U.S.A.), LSD tartrate, apomorphine HCl (Sandoz, Switzerland) and, *d,l*-propranolol (Sigma, U.S.A.) were dissolved in isotonic (0.9%) saline. Ampuls of 2.5 mg/ml of haloperidol (Haldol®, Leo, Sweden) were diluted with saline shortly prior to use. Suspensions of *l*-trans-Δ^9-tetrahydrocannabinol (Δ^9-THC, U.N. Narcotics lab.) contained 10% propylene glycol, 1% Tween-80 and, saline. All drug solutions were freshly prepared and injected intramusculary (IM) in a volume of 1 ml/kg. Doses refer to the forms indicated.

RESULTS

Figure 1 shows the generalization gradients for tests with other doses of *d*-amphetamine (frame A), *l*-amphetamine (frame B), and cocaine (frame C) in the two pigeons (P 27 and P 29) that acquired and maintained the discrimination between 1.6 mg/kg of *d*-amphetamine and saline. The initial key-selection was concordant with the continuing performance throughout the test sessions. The ED_{50} values [22] for *d*-amphetamine, *l*-amphetamine, and cocaine were, respectively: 0.35 mg/kg, 0.55 mg/kg, and 1.05 mg/kg when calculations are based on the average performance of P 27 and P 29. At the individual level, P 27 generalized to lower doses of the test drugs than did P 29.

Figure 2 shows that both pigeons emitted predominantly *d*-amphetamine appropriate responses when the training dose (1.6 mg/kg) of *d*-amphetamine was tested at different occasions 15, 60, and 120 min after the administrations. P 29 emitted mostly vehicle appropriate responses when tested 240 min after the *d*-amphetamine injection whereas P 27 performed *d*-amphetamine responding. Both birds selected the non-drug associated key when testing was carried out 480 min (8 hrs) after the administration of 1.6 mg/kg of *d*-amphetamine. The median effective time interval for the averaged decay of the *d*-amphetamine stimulus is estimated to be 280 min [22].

Figure 3 shows that haloperidol, administered 60 min prior to testing, attenuated the *d*-amphetamine (1.6 mg/kg) stimulus. In frame A, the percentage of responses emitted on the drug associated position out of the total number of pecking responses is illustrated and, frame B shows the initial key selections, i.e., the key on which the bird first completed 15 responses and received the first reward. When based on the averaged %RDP data, the ED_{50} value [22] of haloperidol is estimated to be 0.60 mg/kg. Except for the lower doses of haloperidol tested, the consistency of responding on the initially selected key was variable.

Table 1 lists the results of testing *para*-hydroxyamphetamine, apomorphine, morphine, Δ^9-THC, LSD-25, and propranolol as well as propranolol together with *d*-amphetamine, in the two birds trained to discriminate between the presence and absence of 1.6 mg/kg of *d*-amphetamine. Morphine, Δ^9-THC, propranolol, and *para*-hydroxyamphetamine induced less than 20% of *d*-amphetamine appropriate responding. The dose of 0.125 mg/kg of Δ^9-THC was only tested in P 29 since this bird mostly had not pecked either of the keys when tested with 0.25 mg/kg of Δ^9-THC. LSD-25 and apomorphine produced mixed results. It was only P 27 that once responded with the dose of 0.5 mg/kg of apomorphine. Pretreatments with propranolol (10 and 20 mg/kg) did not reduce *d*-amphetamine (1.6 mg/kg) appropriate responding below 70%.

Throughout the test periods the two pigeons averaged 91% (P 27) and 87% (P 29) correct selections during the *d*-amphetamine training sessions and, 87% (P 27) and 96% (P 29) correct selections during the non-drug, saline training sessions respectively. Generally, the rate of responding was lower during the *d*-amphetamine training sessions as compared to the saline sessions. Usually P 29 evidenced a lower rate than P 27 during *d*-amphetamine sessions but the magnitude of the effect varied across sessions.

DISCUSSION

In general, the present observations in pigeons are congruent with previous studies on the discriminative stimulus properties of *d*-amphetamine in rats meaning that the neurochemical/pharmacological substrates for psychomotor stimulant drug-induced discriminative control are also present in non-mammalian species.

The psychomotor stimulant drugs are interchangeable as regards their discriminative effects in rats and, likewise both *l*-amphetamine and cocaine substituted for *d*-amphetamine in pigeons. In rats, the *d*-amphetamine DS is estimated to be 2 to 5 times more potent than the *l*-isomer stimulus [12, 18, 24, 27], the lowest estimate being closest to the present data. The potency relationship between *d*-amphetamine and cocaine in *d*-amphetamine-trained rats has varied with a factor of 5–6 [4,10] to a factor of 33 [5] and in the pigeons the potency factor is 3 (see also [21]). Anyhow, the commonality in the stimulus effects of these compounds suggests

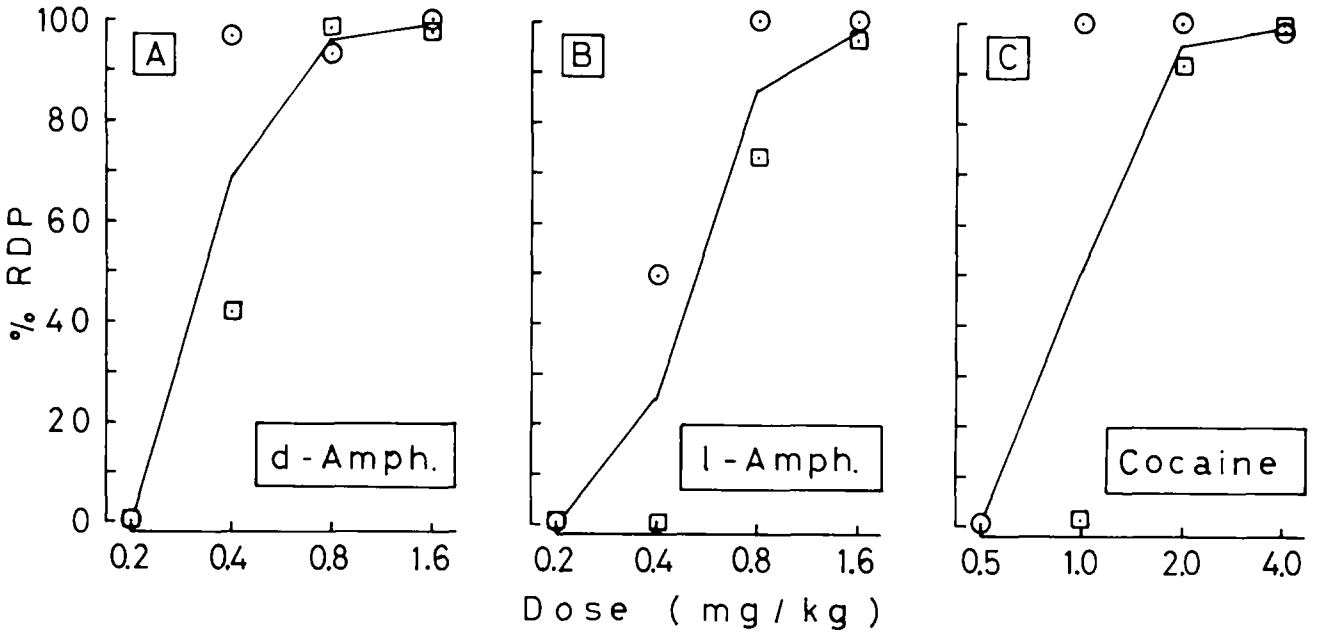

FIG. 1. Generalization gradients for *d*-amphetamine, *l*-amphetamine and cocaine. The two pigeons were trained to discriminate between the presence and absence of effects induced by 1.6 mg/kg of *d*-amphetamine. Abscissa, dose in mg per kg. Ordinate, percent responses on drug (*d*-amphetamine) appropriate position (% RDP). Injection of *d*-amphetamine (d-Amph.) in frame A and *l*-amphetamine (l-Amph.) in frame B were 30 min and *l*-cocaine in frame C was 15 min prior to testing. The curve is based on the mean of four tests, two in each of the birds (P 27: ○ and P 29: □), except for the training dose of *d*-amphetamine (1.6 mg/kg) where 5 sessions are pooled.

TABLE 1

SUBSTITUTION TESTS WITH *PARA*-HYDROXY-AMPHETAMINE (p-OH-Amph.), APOMORPHINE (Apomorph.), MORPHINE, *l*-DELTA-9-TETRAHYDROCANNABINOL (Δ^9-THC), LYSERGIC ACID DIETHYLAMIDE (LSD-25), AND CHALLENGE OF THE *d*-AMPHETAMINE (*d*-Amph.) CUE BY *d,l*-PROPRANOLOL

Drug	Dose (mg/kg)	Time (min)	Number of tests	Responders (%)	% RDP
p-OH-Amph.	1.6	30	4	100	2.2
	3.2	30	4	100	15.6
Apomorph.	0.25	15	4	100	1.8
	0.50	15	4	25	83.5
Morphine	1.5	45	4	100	9.3
	3.0	45	4	25	0.0
	6.0	45	2	0	—
Δ^9-THC	0.125	90	2	100	0.0
	0.25	90	7	43	0.0
	0.50	90	2	0	—
LSD-25	0.04	15	5	80	48.0
	0.08	15	2	0	—
Propranolol	10.0	60	2	100	8.3
+ saline	—	30			
Propranolol	20.0	60	2	100	0.0
+ saline	—	30			
Propranolol	10.0	60	4	100	72.9
+ *d*-Amph.	1.6	30			
Propranolol	20.0	60	4	75	70.4
+ *d*-Amph.	1.6	30			

Responses on *d*-amphetamine position (% RDP) is based upon the performance of the responding birds, i.e., the tests where the animals obtained at least one reinforcement during the test session. Responders refer to the percentage of test occasions where the birds completed at least 15 pecking responses on one of the two keys.

similarities in their mechanisms of action. From previous studies in rats, brain dopamine systems are implicated in mediating the DS of these drugs [11]. In agreement with this proposition the dopamine receptor-blocker haloperidol attenuated the *d*-amphetamine DS in the pigeons; the β-adrenergic blocker propranolol produced only a marginal attenuation of the DS. The doses of haloperidol required to produce the attenuation, however, were rather high and involvement of noradrenergic mechanisms cannot be excluded [2]. Variable responding with haloperidol-amphetamine combinations similar to what was seen in the present study has previously been observed in rats [3,4]. According to Colpaert *et al.* [3] this effect is most properly conceived of as a neuroleptic-produced interference with reinforcement contingencies rather than being a reflection of a limited or partial antagonism between the compounds.

If increased dopaminergic activity constitutes the major component of the *d*-amphetamine DS [11], the dopamine receptor agonist apomorphine ought to be generalized with *d*-amphetamine. Even though this occurred once in one bird with the highest dose of apomorphine there were only 1.8% *d*-amphetamine appropriate responses with 0.25 mg/kg of apomorphine, a dose at which both birds responded on all the tests. A clear-cut generalization from apomorphine to *d*-amphetamine has been reported in three studies [1, 23, 26] whereas partial (40–60%) generalizations were noted by three others [6, 9, 17]. Conversely, in apomorphine-trained rats, tests with *d*-amphetamine have also produced variable results [6,8]. It would appear that the two drugs produce effects in common but the effect-spectra do not overlap completely [25].

That the *d*-amphetamine DS in pigeons is fairly specific is suggested by the lack of generalization with morphine, Δ^9-

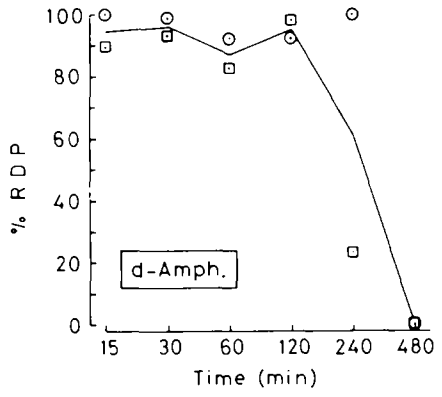

FIG. 2. Duration of the *d*-amphetamine stimulus. The two pigeons were trained to discriminate between the presence and absence of effects induced by 1.6 mg/kg of *d*-amphetamine. Abscissa, time in min between IM injection of 1.6 mg/kg of *d*-amphetamine until testing. Ordinate, percent responses on drug (*d*-amphetamine) appropriate position (% RDP). The curve is based on the mean of four tests, two in each of the birds (P 27: ⊙ and P 29: ⊡) except for the 30 min interval where 3 sessions per pigeon are pooled. The data points were determined on separate days.

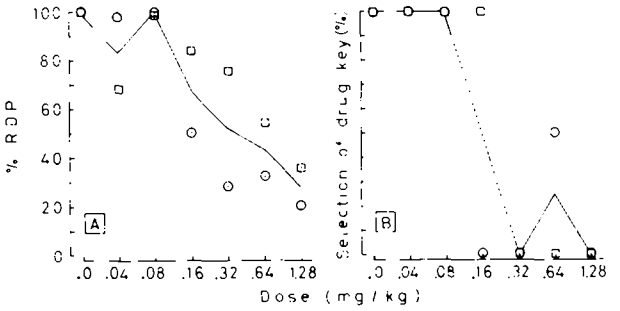

FIG. 3. Combined effects of haloperidol and *d*-amphetamine. The two pigeons were trained to discriminate between the presence and absence of effects induced by 1.6 mg/kg of *d*-amphetamine. Abscissa, frame A and B, dose in mg per kg of haloperidol. Ordinate, frame A, percent responses on drug (*d*-amphetamine) appropriate position (% RDP) and, frame B, percent tests where the birds selected the drug key (selection of drug key), i.e., the key on which the bird initially completed 15 pecking responses and received the first reinforcement. Haloperidol was given 60 min and *d*-amphetamine 30 min prior to testing. Data points are based on one observation in each of the birds except for 0.64 and 1.28 mg/kg of haloperidol where the points represent the average of two tests per animal (P 27: ⊙ and P 29: ⊡).

THC, propranolol, and also LSD. Since injection of both LSD and *d*-amphetamine result in an increased sympathetic activity [14] this commonality in effect may explain the 48% *d*-amphetamine responding observed during the LSD tests.

A central mediation of the present discrimination is indicated by the lack of generalization with *para*-hydroxyamphetamine, a homologue producing effects similar to *d*-amphetamine in the periphery but because of its polar character does not easily penetrate into the CNS [13]. Jones *et al.* [18] reported similar results for rats.

Testing the training dose of *d*-amphetamine at different intervals after injection showed that responding was appropriate for the drug-associated key for 2 hrs in both birds. This interval is longer than what has been reported for rats where *d*-amphetamine appropriate responding is evident 1 hr post-injection (p.i.) and reach the 50% level 1.5 to 2 hrs p.i. [12, 19, 20]. The intraperitoneal route of administration was used in the rat studies whereas the pigeons were given *d*-amphetamine intramuscularly. It would therefore seem possible that this difference in the temporal characteristics of the drug primarily relate to differences in the absorption of the drug, and consequently the elimination of the drug due to differences in the mode of administration rather than reflecting true species specific differences.

This study concludes a series of investigations where drugs (Δ^9-THC, morphine, LSD, and *d*-amphetamine), whose DS properties are relatively well specified in rats, have been established as discriminative stimuli in pigeons ([7, 15, 16], present study). The main focus of these studies has been on species comparisons. To date, no apparent qualitative species differences have emerged regarding the DS of these drugs in rats and pigeons.

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