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BRIEF COMMUNICATION

Repeated Testing Within Drug Discrimination Learning: Time Course Studies With Cocaine, Amphetamine, and 3-PPP

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JÄRBE, T. U. C. *Repeated testing within drug discrimination learning: Time course studies with cocaine, amphetamine, and 3-PPP.* PHARMACOL BIOCHEM BEHAV **44**(2) 481–486, 1993.—Pigeons were trained to discriminate between 3 mg/kg cocaine and saline. Tests with cocaine and amphetamine were conducted at different intervals after administration to compare the time course of the discriminative stimulus (DS) effects. Tests were of two kinds: a) separate, that is, only one dose and interval were examined on each separate test day; and b) repeated, that is, all three intervals were assessed after a single administration of the drug dose during 1 test day. Separate and repeated determinations of the time course yielded similar estimates. The duration of the DS effects of amphetamine were longer than those of cocaine. No apparent difference, either with regard to duration of effect or potency, existed between (+)- and (−)-amphetamine. The potency of cocaine was similar to that of the amphetamine isomers. The dopamine autoreceptor blockers (+)- and (−)-3-(hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP) (1–10 mg/kg) engendered less than 44% cocaine-associated responding for the repeatedly examined intervals (15, 60, and 120 min after administration). The results of this study encourage the use of repeated testing methodology to assess the duration of action of the DS effects of drugs.

Cocaine Amphetamine 3-PPP Drug discrimination Pigeon

MOSTLY only one interval between drug administration and testing is examined to assess discriminative stimulus (DS) effects (21). Although one can probably often predict the correct ("optimal") injection-to-test interval (to coincide with the maximum effect of the drug), previous work suggested that structural changes of the cannabinoid moiety can result in a delay of the onset of effect (22,23).

Thus, tests occurring too early after administration of a slow-acting agent will lead to an underestimation of the drug potency. Examining several intervals after separate administrations is time consuming and may not even be feasible because of a limited supply. To reduce these problems, animals were tested at several intervals after single administrations of Δ-9-tetrahydrocannabinol (Δ-9-THC). The time course estimates so generated compared favorably with determinations where each interval between injection until testing was assessed after a separate dose of Δ-9-THC (13,23). Although the time course of some other compounds have also been evaluated using repeated testing methodologies, no comparative data were offered (12,15,32).

To examine the generality of our previous findings with Δ-9-THC, we compared the time course for some CNS motor stimulants utilizing repeated and separate testing procedures. Pigeons were trained to discriminate between cocaine and saline and then tested for response generalization to isomers of the dopamine releaser amphetamine (30), as well as to isomers of the dopamine autoreceptor blocker 3-hydroxyphenyl-*N*-*n*-propylpiperidine (3-PPP) (11,14).

METHOD

Animals

Four male, mature White Carneaux pigeons with a free-feeding weight of 599 g (SD = 118) were used. These animals

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had participated in another cocaine vs. vehicle discrimination study (20); the present study was carried out through 1982 and 1984. Birds were reduced to about 80% of their free-feeding weights. This weight was maintained by the food presented during sessions and by postsession supplemental feeding. Water and crushed shell grit were always available in the home cages. Between sessions, birds were individually housed in a pigeon colony room (lights in colony room on from 7:00 a.m.–7:00 p.m.; room temperature 20–22°C; relative humidity 50–60%).

Apparatus

Four sound-attenuated and ventilated operant chambers were used. The response keys, 2 cm in diameter and dimly illuminated with white light, were mounted 10 cm apart on the front panel of the chamber, each key 19 cm above the chamber floor. The opening of the key contacts defined a key-pecking response. The food magazine was located between the response keys, 4 cm above the floor of the chamber. The reward was a 4-s access to grain. The key lights went off simultaneously with the 4-s operation of the grain hopper and illumination of food by a magazine light. Conventional relay programming and recording equipment, located in a room adjacent to that of the chambers, were used to control schedule contingencies and record the discrimination performance of birds.

Procedure

Drug discrimination training. The training procedure has been described in detail elsewhere (20). In brief, animals were trained to discriminate between IM administered cocaine HCl and saline. Thus, which key was correct depended upon whether cocaine (3 mg/kg) or vehicle (1 ml/kg) had been administered prior to the session. Birds were placed into their individual experimental chamber immediately after injection and waited there for the 15-min period prior to session onset. The activation of the houselight and illumination of the response keys signaled the start of a session. The schedule of reinforcement used was fixed-ratio 15 (FR 15), that is, the reward was available when 15 key-pecking responses had been accumulated on the key appropriate for the administration [drug (D) or no drug (N)]. Responses on the inappropriate key were also recorded but had no programmed consequences. A training session ended when pigeons had received 52 rewards or 20 min had elapsed since session onset, whichever occurred first. Animals were trained for 3 days/week.

Drug discrimination testing. Animals were tested once a week (Fridays) provided that correct baseline responding was maintained, that is, no more than 29 pecking responses being emitted prior to receiving the first reward during the regular training sessions occurring on Mondays and Wednesdays. Customarily, the order of sessions during test (T) periods was D, N, T, N, D, T, D, N, T, etc. Birds were placed into the experimental chamber immediately after injection and remained there until the session started, when intervals of 30 min or less were examined. Otherwise, birds were returned to their home cages after administration and kept in the pigeon colony room until 15 min remained of the injection-test interval, at which time they were brought to the experimental room and placed into the chamber; 15 min later, the test probe began.

Unlike the regular D and N maintenance sessions, both keys were operable during testing. Thus, pecking on either key produced access to food during each of the six trials comprising a test probe. Like the maintenance sessions, 15 pecking responses had to be accumulated on a key to produce reinforcement. Test probes ended after six food presentations or when 20 min had elapsed since the session onset, whichever occurred first. The order of testing was mixed.

Drugs

Cocaine HCl and both isomers of amphetamine SO_4 (ACO, Sweden) were purchased from the University Hospital of Uppsala, Sweden. The isomers of 3-PPP HCl were generously donated by Dr. H. Selander (ASTRA Alab Co., Södertälje, Sweden). All drugs were dissolved in physiological (0.9%) saline and administered IM (1 ml/kg). Doses refer to the forms indicated.

RESULTS

The percentage of cocaine-appropriate responding of testing two different doses (1 and 3 mg/kg) of cocaine at three different intervals after administration is shown in Fig. 1 (top). Tests were either separate or repeated. Tests with (+)-amphetamine are shown in the lower half of the graph. Results from repeated testing with saline are also shown. The saline tests corresponded to the three intervals examined after cocaine administration (15, 60, and 120 min postinjection), as well as after amphetamine administration (30, 120, and 480 min postinjection). Administration of saline resulted in no drug-appropriate responding throughout both sets of the three test intervals examined. Comparisons within drug [cocaine (top) and amphetamine (bottom)] suggest that repeated and separate testing produce similar results both with regard to duration of effect and potency. Comparisons across the two drugs suggest that (+)-amphetamine has a longer duration of action than that of cocaine and that the two drugs are equipotent.

Results from repeated tests with (−)-amphetamine are listed in Table 1. The data suggest that (−)-amphetamine is equipotent to (+)-amphetamine and cocaine. Further, the duration of action of the discriminative stimulus effects of (−)-amphetamine is similar to that of (+)-amphetamine and, consequently, longer than that of cocaine. The response rate data for (−)-amphetamine are also listed in Table 1. A response pattern similar to that seen with the (−)-isomer was observed also for (+)-amphetamine, as well as cocaine, that is, the highest doses decreased the rate during the first test interval (not shown).

Neither of the isomers of 3-PPP engendered more than 44% cocaine-appropriate responding. The highest doses tested produced marked rate suppression, that is, for each isomer only three birds received at least one reinforcement during the first test interval. Also, for birds that did receive reinforcement the response rate was lower than their corresponding rate during the nondrug maintenance sessions immediately preceding these test sessions (cf. rate in Table 1). The dose-related decrease of rate by both isomers of 3-PPP had recovered during the third test interval, occurring 2 h after administration.

During periods of testing, the four birds performed correctly 94.0, 100, 91.2, and 96.7%, respectively, during cocaine (3 mg/kg) maintenance sessions; these individual data are based upon a total of 251 D sessions (mean ± SEM: 62.8 ± 3.5). The corresponding values for the nondrug maintenance sessions were, respectively: 100, 100, 100, and 97.0% (data

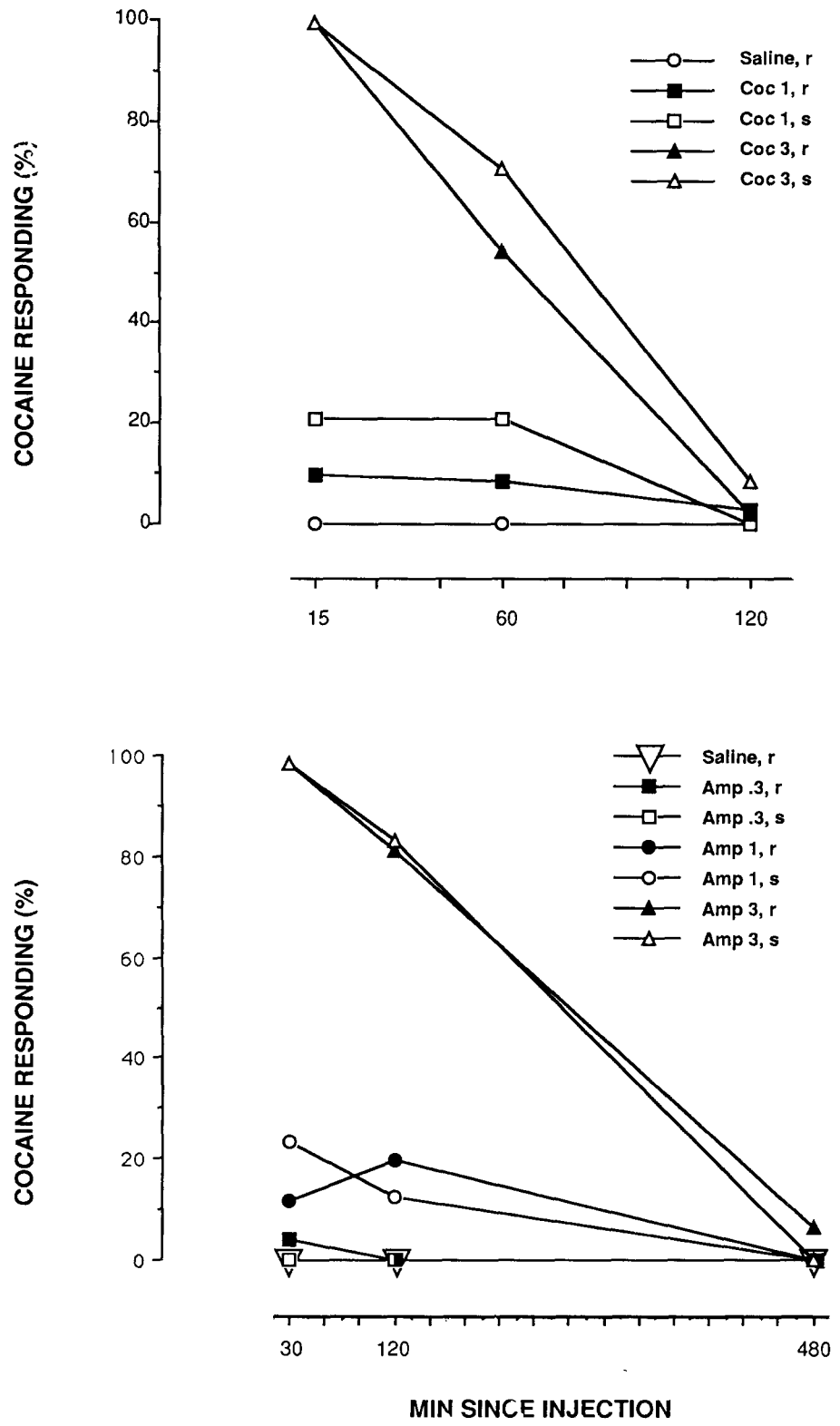

FIG. 1. Time-course determinations with cocaine (Coc, top) and (+)-amphetamine (Amp, bottom) utilizing separate (s) and repeated (r) testing procedures for pigeons trained to discriminate between the effects produced by cocaine (3 mg/kg) and saline (1 ml/kg). *y*-axis, percentage of cocaine-appropriate responding; *x*-axis, time intervals in minutes since administration. Data points represent the mean of one to two determinations for each of the four birds.

TABLE 1

TIME COURSE OF THE DISCRIMINATIVE STIMULUS EFFECTS OF (−)-AMPHETAMINE, (−)-3PPP, AND (+)-3PPP AS EVALUATED BY REPEATED TESTING

Drug	Dose (mg/kg)	Time (min)	*n/n*	Rate (±SEM)	% RDP
(−)-Amp	0.3	30	8/8	0.94 (0.11)	12.5
	1.0	30	8/8	0.85 (0.04)	37.5
	3.0	30	7/7	0.69 (0.38)	87.5
(−)-Amp	0.3	120	8/8	0.92 (0.11)	12.5
	1.0	120	8/8	0.90 (0.02)	17.5
	3.0	120	7/7	0.58 (0.09)	80.3
(−)-Amp	0.3	480	8/8	1.03 (0.03)	0.0
	1.0	480	8/8	0.97 (0.03)	4.2
	3.0	480	7/7	1.17 (0.27)	10.1
(−)-3PPP	1.0	15	4/4	1.01 (0.09)	4.2
	3.0	15	4/4	0.92 (0.15)	0.0
	10.0	15	3/4	0.36 (0.29)	1.4
(−)-3PPP	1.0	60	4/4	1.17 (0.05)	0.0
	3.0	60	4/4	0.97 (0.06)	0.0
	10.0	60	4/4	0.68 (0.19)	20.8
(−)-3PPP	1.0	120	4/4	1.05 (0.10)	0.8
	3.0	120	4/4	1.03 (0.18)	0.0
	10.0	120	4/4	0.94 (0.12)	23.1
(+)-3PPP	1.0	15	4/4	1.09 (0.10)	2.1
	3.0	15	4/4	0.55 (0.36)	16.7
	5.6	15	3/4	0.46 (0.21)	25.0
(+)-3PPP	1.0	60	4/4	1.05 (0.11)	12.8
	3.0	60	4/4	0.69 (0.24)	20.3
	5.6	60	4/4	0.54 (0.24)	43.9
(+)-3PPP	1.0	120	4/4	1.06 (0.15)	24.5
	3.0	120	4/4	0.93 (0.14)	2.6
	5.6	120	4/4	0.79 (0.15)	33.3

Time in minutes since administration until testing. *n/n*, number of responding birds/number of tests; a responder obtained at least one reinforcement of six possible during the 20-min test probe. Rate (mean ± SEM) is the quotient between the time taken to complete the cycle for the initial six rewards during nondrug (saline) maintenance sessions divided by the time taken to obtain the six rewards during a test probe; values below 1 signify longer times and values above 1 indicate shorter times to complete the six-trial cycle during test probes. SEM is calculated as SD divided by the inverse square root of the sample size minus 1; only responders were included in these calculations. % RDP, percentage of pecking responses directed toward the drug (cocaine)-associated key of the total number of responses emitted during a test probe. The data represent the average % RDP from one to two determinations for each responding bird (N = 4). Technical error reduced the number of observations to seven for 3 mg/kg (−)-amphetamine, that is, only one observation is available for one of the four birds.

based upon a total of 262 N sessions; mean ± SEM: 65.5 ± 3.6).

DISCUSSION

The present investigation indicates that orderly time course estimates of the DS effects of CNS motor stimulants can be obtained using a repeated testing strategy. Thus, the time courses for cocaine and amphetamine were similar irrespective of whether the data points were obtained after each dose of the compounds was assessed on separate test days or when the three time intervals were determined after one administration of a particular dose during a single test day. These results further the conditions under which repeated testing methodology can be utilized to determine the duration of the DS effects of drugs.

The duration of the DS effects of cocaine disclosed a decline during tests 1 h after administration, and little cocaine-appropriate responding occurred during tests conducted 2 h after injection. This is in agreement with previous studies with pigeons (9,18). The decay of the cueing effects of cocaine for pigeons is in reasonable accord also with studies using rats (17,27,29).

In tests for response generalization, amphetamine substituted for cocaine. This is in agreement with previous findings using various species (5,8,9,10,18). The duration of action for the DS effects of amphetamine seems longer for pigeons [this study; (19)] than for rats (16,24,26). Apart from the possibility that this reflects a true species difference, the seeming discrepancy may relate to the route of administration employed. For pigeons, the IM route of administration is commonly employed whereas for rodents the IP route of administration is mostly used. Species comparisons involving different routes of administration will have to be conducted to settle this issue.

Cocaine and the amphetamine isomers did not disclose any major differences with regard to potency. For the discriminable as well as for other measures, the potency order commonly has been found to be (+)-amphetamine > (−)-amphetamine > cocaine in rats (2,5,6,16,28,31). For two pigeons trained to discriminate between (+)-amphetamine and saline, slight indications for such an order of potency was previously found (19). Nonetheless, it would seem that the differences in potency between these compounds are less for pigeons than for rats. The potency differences between *d*-amphetamine and cocaine in primates are also rather small (7,10,25). However, as pointed out above, route of administration may be an important determinant for these species differences.

The dopamine autoreceptor blockers (+)- and (−)-3-PPP did not substitute for cocaine during any of the three intervals examined. For both isomers, doses were included that markedly affected the rate of responding. Because of the limited experience with these compounds within drug discrimination, the inclusion of several test intervals in this study render validity to these negative results and further attest to the specificity of the cocaine cue in pigeons. For rats trained to discriminate between the purportedly D_2 receptor agonist LY 171555 and vehicle, tests with (−)-3-PPP disclosed complete response generalization; tests with "indirect" dopamine agonists such as amphetamine and cocaine failed to substitute (1). This is congruent with the present results. The failure of 3-PPP to substitute for cocaine would suggest that 3-PPP produces stimulus effects different from those induced by the CNS motor stimulants cocaine and amphetamine for both rats and pigeons. Thus, dopamine autoreceptor blockade is not sufficient to elicit cocaine-like DS effects.

Birds made slightly more errors in selecting the appropriate key during cocaine as compared to saline maintenance sessions. This is in agreement with previous data for these pigeons (20), although the difference between the two training conditions (i.e., cocaine and saline) was less in the present study. Studies by Colpaert and colleagues (3,4) suggested that rats may exhibit a bias in the opposite direction, that is, a bias toward the cocaine lever. Thus, rats disclosed more errors when trained with saline rather than when trained with cocaine. Witkin et al. (31), however, reported a fairly even distribution of errors for rats during cocaine and saline maintenance sessions. Thus, pigeons and rats do not necessarily disclose a species-specific predominance for committing certain types of errors when trained for cocaine vs. saline discrimination.

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