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Effects of d-Amphetamine and Chlordiazepoxide on Positive Conditioned Suppression[1]

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POLING, A., C. URBAIN AND T. THOMPSON. *Effects of d-amphetamine and chlordiazepoxide on positive conditioned suppression.* PHARMAC. BIOCHEM. BEHAV. 7(3) 233–237, 1977. – Six rats lever-pressed under a variable-interval 80-sec food reinforcement schedule. After responding had stabilized, an 8-sec tone terminating with food delivery was superimposed on the variable-interval schedule on the average once every five minutes without regard to the animal's behavior. This positive conditioned suppression procedure consistently reduced responding during the pre-food stimulus (tone). Neither *d*-amphetamine (0.5, 1.0, 2.0 mg/kg) nor chlordiazepoxide (7.5, 15, 30 mg/kg) significantly affected the relative suppression produced by the tone. Instead, both drugs produced generally non-selective effects, similarly affecting response rate in the presence and absence of the tone.

Positive conditioned suppression Operant behavior *d*-Amphetamine Chlordiazepoxide

THIRTY-SIX years ago, Estes and Skinner [7] demonstrated that the food-maintained responding of rats was suppressed during the presence of a brief tone terminating with delivery of an unavoidable shock. Subsequent studies extended this finding across species and reinforcement schedules (e.g., [1, 3, 6, 16]). The Estes-Skinner procedure rapidly attracted psychopharmacological interest because the suppression during the pre-shock stimulus was assumed to result from anxiety [7], an assumption since criticized on various grounds [6, 9, 16]. Despite such criticism, the effects of various compounds on responding suppressed by the Estes-Skinner procedure have been reported (e.g., [3, 6, 11, 16, 20]). Unfortunately, perhaps due to wide differences in experimental parameters, results have been somewhat inconsistent [6, 9, 16]. In general, it has been reported that acutely-administered amphetamines do not increase responding in the presence of a pre-shock stimulus, although acutely-administered benzodiazepines may do so [11, 14, 16].

A procedure known as positive conditioned suppression, in which a brief stimulus that precedes food delivery is occasionally superimposed on a baseline of operant responding, may also suppress responding during the prefood stimulus [2, 13, 14, 15]. This procedure resembles the Estes-Skinner procedure in that each involves the superimposition of respondent contingencies on an operant baseline, and each results in suppression of ongoing operants. In both cases, this suppression seems to primarily involve the evocation of behaviors incompatible with the required operant, although the specific behaviors evoked by preshock and prefood stimuli differ [2, 13, 14, 16]; the Estes-Skinner procedure may also involve superstitious punishment resulting from an aversive stimulus (shock) fortuitiously following responding.

Considering the research generated by the Estes-Skinner procedure, and the known similarities and differences between the Estes-Skinner and positive conditioned suppression procedures, an examination of drug effects during positive conditioned suppression is of interest. A single study [14] has examined drug effects during positive conditioned suppression. Miczek found that the relative suppression produced by a prefood stimulus was unaffected by chlordiazepoxide, diazepam, and scopolamine, but was reduced by d-amphetamine [14]. However, in light of the sensitivity of positive conditioned suppression to parametric changes [13,15], and the demonstrated effects of procedural variations on drug effects under the Estes-Skinner procedure (e.g., [6, 9, 16]), it is possible that these findings may be limited to the particular experimental parameters studied by Miczek.

The present study explored this possibility by determining the effects of acute administration of d-amphetamine and chlordiazepoxide under a positive conditioned suppression procedure paradigmatically similar to that

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studied by Miczek. However, the studies differed in a number of parameters including baseline reinforcement schedule, deprivation level under which subjects were tested, length and frequency of presentation of the prefood stimulus, and (in some cases) drug doses given. Each of these variables has been shown to influence drug effects under the Estes-Skinner procedure.

METHOD

Animals

Six adult male Sprague-Dawley rats maintained at approximately 85% of free-feeding weights ($\bar{X}$ weight = 425 g) were used. They were individually housed in a constantly-illuminated room with an ambient temperature of 24° C.

Apparatus

Each of three sound-attenuated Gerbrands operant conditioning chambers was equipped with two levers and a pellet dispenser. A 7-W white light located in the feeder opening and a 25-W white house light supplied constant ambient illumination. An exhaust fan provided ventilation and masking noise. Noyes food pellets (45 mg) were presented following designated presses of the left lever. A force of approximately 0.2 N was required for lever operation. Presses of the right lever had no programmed consequences. Electromechanical programming and recording equipment was located in another room.

Procedure

Each animal was initially trained to press the left lever for food pellets available under a fixed-ratio 1 (FR 1) schedule of reinforcement. Under this schedule, each lever press produced food. After five one-hr sessions at FR 1, the reinforcement schedule was changed to a variable-interval 5-sec schedule (VI 5-sec) which was gradually lengthened across fifteen sessions to VI 80-sec. Under this schedule, a pellet was delivered following the first response emitted after an irregular temporal interval ($\bar{X}$ = 80 sec, range = 4-240 sec) had elapsed since delivery of the preceding pellet. Each animal was exposed to the VI 80-sec schedule for 30 one-hr sessions. A single session for each animal was conducted daily, at approximately the same time each day.

After the thirtieth VI 80-sec session, an 8-sec tone terminating with delivery of a food pellet was programmed to occur under a variable-time 5-min (VT 5-min) schedule during each session. Under this schedule, the tone was presented on the average once every five min (range = 30 sec – 15 min) without regard to the animal's behavior. The tone and food were introduced together since previous research [18] indicated that tone presentation alone did not affect lever pressing. Superimposition of the tone on the VT 5-min schedule constituted the positive conditioned suppression procedure. The VI 80-sec schedule, which remained in effect for all sessions, cycled independently of the VT 5-min schedule so that food availability on the VI 80-sec schedule was not affected by the occurrence of the tone.

After ten sessions of exposure to the positive conditioned suppression procedure, total responses per session in the presence of the tone showed no obvious trend across sessions. At that time, an injection regimen was begun in which each animal received 0.0, 0.5, 1.0, or 2.0 mg/kg d-amphetamine, or, after completion of the d-amphetamine series, 0.0, 7.5, 15, or 30 mg/kg chlordiazepoxide intraperitoneally thirty min prior to each session. Either d-amphetamine or chlordiazepoxide was given prior to every fourth session, with 0.9% sodium chloride injections preceding all other sessions. Each animal received each drug dose two times, in an irregular order. Both d-amphetamine sulfate and chlordiazepoxide hydrochloride were dissolved in 0.9% sodium chloride solution prepared so that the total volume injected was always 1.0 ml/kg.

During each session, number of responses during the tone, number of responses during the 8-sec period preceding each tone, and number of responses during the rest of the session were recorded separately. A repeated measures analysis of variance was used to evaluate the data, and mean drug and nondrug (control) response rates were compared by the Newman-Keuls method [10]. Unless otherwise indicated, all probability statements refer to Newman-Keuls comparisons. Suppression ratios [12] were also calculated for each prefood stimulus and evaluated as above. The suppression ratio equals: responses during the 8-sec tone presentation / responses during the 8-sec tone presentation + responses during the 8-sec interval preceding tone presentation. This ratio is less than 0.50 when the tone suppresses responding, 0.33 when the tone suppresses responding by 50%, and 0.00 when suppression is complete.

RESULTS

Figure 1 shows mean responses per minute by all animals during the presence and absence of the tone under all experimental conditions. When the rates of all animals are averaged, an intermediate response rate occurred during baseline sessions when saline injections were given. However, appreciable differences in response rates occurred across animals, although the response rate of an individual animal was relatively stable across baseline sessions. During the tone, mean response rate for the group of animals fell by about fifty percent. The mean decrease in response rate produced in individual animals ranged from 27% to 73%.

Administration of both d-amphetamine and chlordiazepoxide produced dose-dependent effects. For each drug, mean group response rate in the absence of the tone slightly increased at the lowest dose tested and progressively decreased at the higher doses. However, these effects were statistically significant ($p<0.05$) when compared to the non-drug rate only at the highest doses given. In the presence of the tone, d-amphetamine produced dose-dependent decreases in mean group response rate, with the decrease being statistically significant ($p<0.05$) at 2.0 mg/kg. The lowest dose of chlordiazepoxide (7.5 mg/kg) slightly increased the mean group response rate during the tone relative to the non-drug rate, although this increase was not statistically significant at the 0.05 probability level. The higher doses of this drug progressively decreased mean group rate of responding in the presence of the tone, with this effect being statistically significant ($p<0.05$) at the 30 mg/kg dose.

For each drug across all animals, a positive correlation (Pearson r, [10]) occurred between the number of responses emitted during the 8-sec tone presentation and the number of responses emitted during the 8-sec interval preceding the tone. For d-amphetamine, the correlation was 0.51 (df = 522, $p<0.01$), while for chlordiazepoxide the correlation

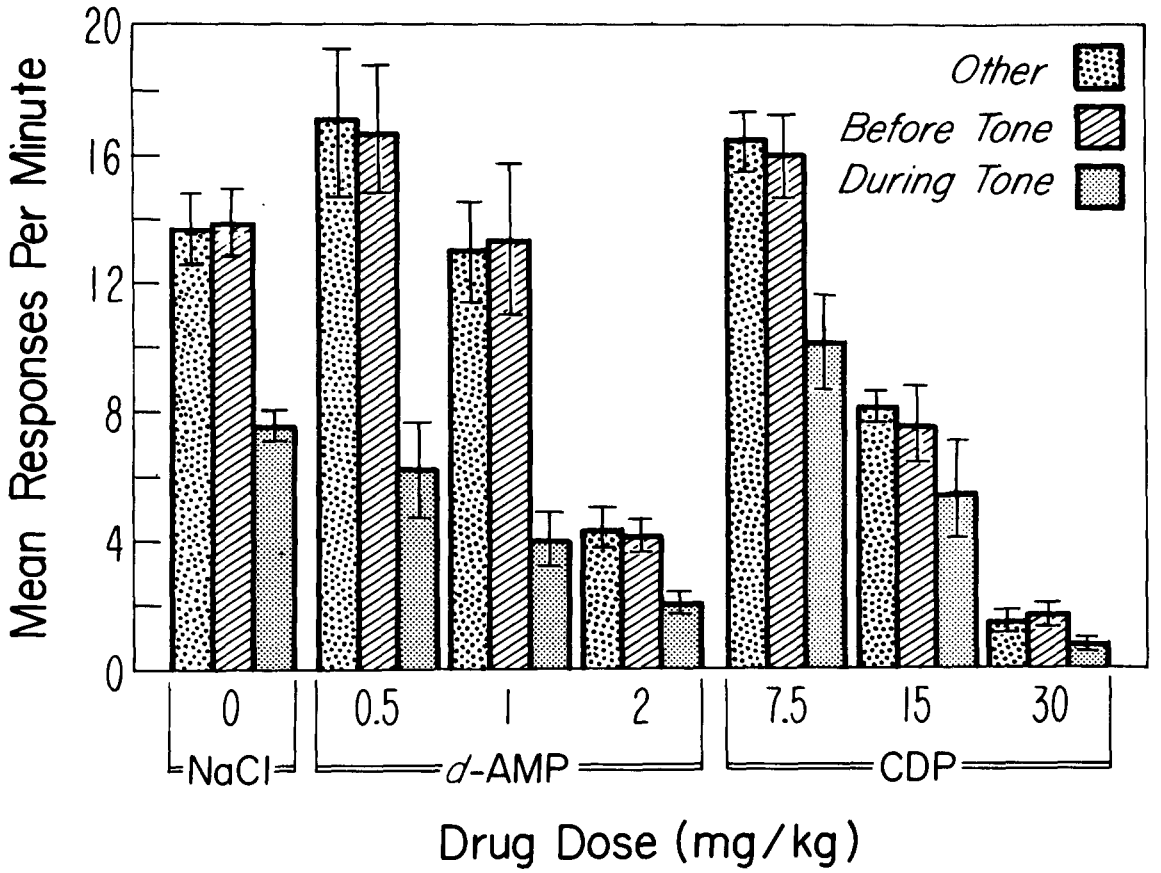

FIG. 1. Mean responses per minute during the presence and absence of the prefood stimulus (tone) under all experimental conditions. Responses during the absence of the tone are divided into responses during the 8-sec interval preceding each tone (before tone) and responses during the rest of the session (other). The former measure provides a means of evaluating sampling error resulting from recording data during intervals equal in length to tone presentations. Since mean rates during the pre-tone interval closely parallel mean rates during the rest of the session in the absence of the tone, these measures can be considered as equivalent. Baseline (NaCl) values represent the mean for six animals across 36 sessions while each drug mean represents two sessions for each animal. The area within brackets is ± 1 standard error.

was 0.47 (df = 522, $p<0.01$). These correlations suggest that drug effects were relatively non-selective: Across all drug administrations, responding during the presence and absence of the tone was similarly affected.

Mean group suppression ratios are shown in Table 1. This measure also indicates that drug effects were non-selective: Neither drug significantly ($p<0.05$) affected the mean group suppression ratio at any dose tested, although all doses of d-amphetamine slightly decreased the mean suppression ratio while all doses of chlordiazepoxide slightly increased it.

DISCUSSION

Under the conditions of the present study, neither d-amphetamine nor chlordiazepoxide significantly attenuat-

TABLE 1

SUPPRESSION RATIOS UNDER ALL EXPERIMENTAL CONDITIONS. RATIOS LESS THAN 0.50 INDICATE SUPPRESSED RESPONDING IN THE PRESENCE OF THE PRE-FOOD STIMULUS (TONE). INCREASES IN THE SIZE OF THE SUPPRESSION RATIO COMPARED TO THE BASELINE VALUE INDICATE ATTENUATED RELATIVE SUPPRESSION. SEE TEXT FOR AN EXPLANATION OF THE SUPPRESSION RATIO AND EXPERIMENTAL CONDITIONS

		Drug					
	Saline (Baseline)	d-Amphetamine			Chlordiazepoxide		
mg/kg	—	0.5	1	2	7.5	15	30
Mean Suppression Ratio	0.35	0.27	0.28	0.33	0.40	0.44	0.36
Standard Error	0.04	0.05	0.06	0.04	0.03	0.05	0.04

ed the absolute or relative response suppression produced by a prefood stimulus. Both drugs generally produced non-selective effects, similarly affecting response rate in the presence and absence of the prefood stimulus. The failure of chlordiazepoxide to attenuate the suppression produced by a prefood stimulus is consistent with previous findings [14]. However, Miczek [14] reported that d-amphetamine increased response rate during a prefood stimulus. He also reported no significant correlation between responding during the presence and absence of the prefood stimulus, which is contrary to the results of the present study.

The factors accounting for the differences in the results of the two studies are unclear. The present study and that of Miczek [14] represent the only attempts at evaluating drug effects on responding suppressed by response-independent presentations of a non-aversive stimulus (i.e., food). The studies differed with respect to a number of parameters, which were apparently sufficient to produce differential drug effects.

Previous studies utilizing the Estes-Skinner procedure have demonstrated that experimental parameters directly influence drug effects. For instance, depending on the parameters studied, d-amphetamine may increase [21], decrease [4], or have no effect on [11,14] responding during a preshock stimulus. Many of the factors influencing drug effects under the Estes-Skinner procedure have been determined. One factor of importance is response rate. Wuttke and Kelleher [24] contended that drug effects on responding suppressed by a preshock stimulus depend critically on the control rate of responding during the stimulus, although others [5] have argued that simple rate-dependency does not fully account for drug effects under this procedure. Sanger and Blackman [22] have also noted that response rate in the absence, as well as in the presence, of a preshock stimulus may be important in determining drug effects on the relative suppression produced by a preshock stimulus as measured by the suppression ratio. In their study, decreases in relative suppression produced by d-amphetamine resulted primarily from decreases in response rate in the absence of the preshock stimulus.

Overall control response rates during both the presence and absence of the prefood stimulus were considerably lower in the present study than in the Miczek study [14]. The absolute difference between overall response rates during the presence and absence of the prefood stimulus was also greater in the Miczek study (where the difference was approximately 39 responses per min) than in the present study (where the difference was about 7 responses per minute). Finally, local response rates during the prefood stimulus were not obtained in either study. Local, rather than overall, response rates are of primary importance in determining drug effects on responding maintained by response-independent food delivery [19], as well as by conventional reinforcement schedules. This dependence of drug effects on local response rate obtains even when the scheduled reinforcers are as intuitively different as electric shock and food (e.g., [17]). In view of these considerations, it is likely that differences in control response rates were at least partially responsible for the differences between the drug effects reported by Miczek [14] and those found in the present study.

A second factor possibly accounting for these differential effects is the drug injection procedure itself. Miczek used an intermuscular route of administration and injected drugs five min prior to 45-min sessions; in the present study, drugs were injected intraperitoneally thirty min before 1-hr experimental sessions began. Blood levels obtained across a session with a particular drug dose were undoubtably affected by these differences. Responding under a variable-interval schedule in the absence of the prefood stimulus was surely less sensitive to a given drug dose in theMiczek study than in the present study: Miczek reported no significant drug effects on such responding with chlordiazepoxide doses as high as 40 mg/kg. Similar doses have been previously reported to produce behavioral depression and ataxia (e.g., [8, 21, 23]) and did so in the present study.

Results of the two studies which have evaluated drug effects under a positive conditioned suppression procedure suggest that such effects are dependent on the specific procedure used. As is the case in the Estes-Skinner procedure [6, 9, 16], a number of factors may interact to produce various drug effects under paradigmatically similar positive conditioned suppression procedures. However, as Millenson and Leslie [16] pointed out in a recent review, it is possible to empirically determine the factors controlling drug effects under the Estes-Skinner procedure. When these factors are held constant, drug effects are reliable and may be readily interpreted within existing conceptual frameworks. It seems probable that drug effects under a positive conditioned suppression procedure are similarly lawful; however, empirical determination of the factors controlling such effects remains a task for future research. It is presently premature to speculate whether drug effects systematically differ for behaviors suppressed by response-independent delivery of aversive (i.e., shock) and non-aversive (i.e., food) stimuli (cf., [14]).

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