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Discriminative Stimulus Effects and Antipunishment Effects of Drugs Measured During the Same Session

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McMILLAN, D. E., M. LI AND W. C. HARDWICK. *Discriminative stimulus effects and antipunishment effects of drugs measured during the same session.* PHARMACOL BIOCHEM BEHAV **56**(2) 161–166, 1997.—The effects of pentobarbital, diazepam, phencyclidine, buspirone and methamphetamine on drug discrimination and on responding under a variable-interval variable-interval with punishment schedule were studied in pigeons trained to discriminate 5.0 mg/kg pentobarbital from saline. Pentobarbital produced dose-dependent increases in the proportion of responses on the drug key and on rates of punished responding. Diazepam had very similar effects except that the dose-effect curve for punished responding turned over at the highest dose level. Phencyclidine produced only partial responding on the drug key and weakly increased punished responding. Buspirone produced small increases in punished responding, but in the drug discrimination experiments buspirone did not cause responding on the drug key. Methamphetamine did not produce responding on the drug key, nor did it increase rates of punished responding. These experiments are among the first to demonstrate that drug discrimination and other behaviors can be studied within single test sessions in the same animals and they suggest that there is a close correspondence between the discriminative stimulus effects of some drugs and their anti-punishment activity. **Copyright © 1997 Elsevier Science Inc.**

Drug discrimination Methamphetamine Punished responding Pigeons Pentobarbital Buspirone Diazepam Phencyclidine

WE have argued previously that there is a relationship between the effects of arylcyclohexylamines on punished responding and their discriminative stimulus properties (9). The basis of this argument is that the affinity of a number of arylcyclohexyl amines for the phencyclidine receptor correlates highly with both the relative potency of these drugs as discriminative stimuli and their potency as drugs that increase punished responding. This would suggest that both the discriminative stimulus properties of these drugs and their antipunishment effects are mediated through common, or similar populations of phencyclidine receptors.

It is possible that there might be a close relationship between the discriminative stimulus properties of other anti-anxiety drugs and their anti-punishment activity. To study this possibility, a group of pigeons that had been trained previously to discriminate 5 mg/kg pentobarbital from saline, also learned to respond for food under a multiple variable-interval variable-interval with punishment (mult VI VI pun) schedule of food presentation, during which every 5th response under the VI pun schedule was punished with electric shock. Training to respond for food under the mult VI VI pun schedule occurred two days per week, while the remaining days were devoted to maintaining the drug discrimination.

After responding stabilized on these two schedules, dose-response curves were determined for the effects of pentobarbital and other drugs on responding under both the drug discrimination schedule and under the mult VI VI pun schedule. Pentobarbital was chosen because it produces large increases in punished responding (7) and is rapidly established as a discriminative stimulus during differential reinforcement (11). Diazepam was chosen because it also produces large increases in punished responding (7) and because in animals trained to discriminate pentobarbital from saline, there is complete generalization to diazepam (12). Phencyclidine was chosen because animals trained to discriminate phencyclidine from saline show some responding on the drug key after pentobarbi-

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tal administration (10) and phencyclidine weakly increases punished responding (13). Buspirone was chosen because it increases punished responding in pigeons (9,14), but does not show discriminative stimulus generalization to barbiturates (1). Methamphetamine was chosen because it neither increases punished responding (7), nor shows discriminative stimulus generalization to the pentobarbital stimulus (11).

METHODS

Subjects

Four male White Carneaux pigeons (Palmetto Pigeon Plant, Sumter, SC), P-251, 253, 254, 327 served as experimental subjects. These birds had previously been used for studying the effects of food deprivation on sensitivity of the discriminative stimulus properties of pentobarbital. They were individually housed with free access to food and water in a temperature- and humidity-controlled room that was maintained under a 12-h normal phase lighting cycle. The birds had been maintained at 80% (400-485 grams) of their free-feeding weights in the previous experiments and were at the 80% weights in the current experiment.

Apparatus

The experimental chamber was a Gerbrands Model G5610 (Gerbrands Corp., Arlington, MA) pigeon test cage equipped with three response keys, each of which could be transilluminated with several colors by a Gerbrands 28-V dc key light assembly containing two 0.04-W bulbs for each color. A food hopper (Gerbrands) containing mixed grain was accessable to the pigeon when scheduled contingencies were met. The chamber was enclosed inside a Gerbrands Model G7211 sound- and light-attenuating enclosure. A relay mounted inside the chamber operated whenever the key contacts were opened to provide auditory feedback for responses. A houselight illuminated the experimental chamber during the session except during a feed cycle when a light over the food hopper was illuminated. Electric shock (200 msec duration, 120 V a.c., 60 Hz, mA individualized to the bird) was delivered to stainless-steel electrodes implanted around each pubis bone (2). The birds were connected to the shock source via a plug attached to a leather harness which the bird wore at all times. The impedance of the electrodes was measured daily to ensure constancy of stimulus presentation. Schedule contigencies and data collection were programed by a microcomputer (Gateway 2000) through an interface (MED Associates, Inc., East Fairfield, VT). The microcomputer was housed in a room adjacent to the room containing the test chamber.

Procedure

Training procedure. The birds had been trained previously to discriminate a 5 mg/kg of pentobarbital from saline using the color-tracking under second-order schedules (8) and then were used in experiments to study effects of different levels of food satiation on drug discrimination. Under the second-order schedule of reinforcement in effect for these experiments, a single peck on a white center key extinguished it and lighted the two side keys, one with a red light and one with a green light. Completion of five responses on either side key (fixed-ratio 5, FR5) extinguished the side keys, reset the ratios on the side keys to 5, and relighted the center key to reinstate the original condition. Food was presented for 8 sec only after ten FR 5 units had been completed on the "correct" side key. This schedule has been referred to as a second order FR 10 (FR 5) schedule, as the birds are required to complete 10 FR 5s on the correct key to produce the food reinforcer (8). If 5.0 mg/kg pentobarbital had been administered 10 min before the session, pecks on the red key were defined as correct and produced food under the FR 10 (FR 5) schedule. If saline had been administered 10 min before the session, pecks on the green key were defined as correct. Five pecks on the key not defined as correct relighted the center key, but did not decrease the number of FR 5s required on the correct key for food delivery. Position of the red and green colors on the side keys varied randomly after each center-key response. On training days, the session terminated after food presentation had occurred ten times , or after 900 s, whichever occurred first. Sessions were conducted 7 days per week.

After stimulus control for drug discrimination under the color-tracking schedule was established, the training nonpunishment-punishment procedure was initiated. The birds were trained to peck the center key under a multiple variable-interval variable-interval schedule with nonpunishment and punishment components (mult VI VI punishment). In the nonpunishment component, responding of the birds was maintained under a VI 90-sec schedule of food presentation. In the punishment component, responding of the birds also was maintained under VI 90 schedule, but every 5th response produced an electric shock. Sessions began with the nonpunishment component and components alternated until a total of 6 components had been presented; 3 presentations of the nonpunishment component and 3 presentations of the punishment component. To distinguish the two different components of the schedule, a blue key light was associated with the nonpunishment component and a yellow key light was associated with the punishment component. The electrical shock used to suppress behavior was adjusted for each bird during punishment periods. All birds began punishment training with shock intensity at 1.0 mA. It was then adjusted, in steps of 0.25 mA, for each animal independently to maintain a level of responding during punishment components at about 50% of the unpunished response rate. Final shock levels ranged from 2.5 to 3.5 mA. The sessions lasted for 30 min and the birds were given daily sessions for several weeks.

After a stable responding under the multiple VI 90, VI 90 (punishment) schedule was developed, a training procedure of drug discrimination sessions and mult VI VI punishment sessions started with the following sequence: DSVDSV [D = Drug administration day (drug discrimination training), S = Saline administration day (drug discrimination training), V = Mult VI VI punishment session day]. This training sequence repeated until the subjects reached the stability criteria such that the percentage of responding on the correct key was equal to or greater than 80% during six consecutive discrimination training sessions and the base line rate of unpunished responding was stable with the rate of punished responding at approximately 50% of the unpunished rate.

Test procedure. Test sessions began when a subject reached the training criteria described above. The test session consisted of two phases. In the first phase of the session, drug discrimination was measured with various doses of drugs (pentobarbital, diazepam, phencyclidine, buspirone and methamphetamine) being substituted for the training dose of pentobarbital. In the second phase of the session, the effects of these same drugs and doses on unpunished responding and punished responding were determined. To summarize briefly, a bird was injected intramuscularly and placed into the chamber. After a 10 minute presession delay, the first phase was initiated and it was

terminated with the first food delivery, or 10 min, whichever occurred first. Food was delivered upon the completion of 10 FR 5s on either the red or the green key, whichever occurred first. Immediately after food delivery or expiration of the 10 min session, the second phase began with the multiple VI 90, VI 90 (punishment) schedule in effect. The second phase lasted 30 minutes. Drug tests were conducted on Tuesdays and Fridays. Thursdays served as vehicle sessions and were used to estimate base-line variability. The data used in the construction of dose-effect curves were the result of a single test session on a given day at each dose for each pigeon.

Data Analysis

Discrimination data from the first phase of the test sessions were plotted as a percentage of responses on the red key (hereafter referred to as the pentobarbital key). The average rate of responding on the side keys was also plotted. Data from the second phase of the test sessions were determined to measure the effects of drugs on punished and unpunished responding. The response rates after drug administration were compared to those after administration of drug vehicle (Thursday's sessions). Drug effects falling more than two standard deviations from the Thursday vehicle means were considered to be statistically significant.

Drugs

Pentobarbital sodium (Sigma Chemical Co., St. Louis, MO), Phencyclidine (PCP) hydrochloride (National Institute on Drug Abuse, Rockville, MD), Methamphetamine hydrochloride (Sigma Chemical Co., St. Louis, MO) and Buspirone hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% physiological saline, and diazepam (Elkins-Sinn, Inc., Cherry Hill, NJ) was dissolved in diazepam solvent (40% propylene glycol, 10% alcohol and 50% saline) to concentrations allowing an injection volume of 1 ml/kg and administered intramuscularly into a breast muscle. Physiological saline and diazepam solvent were also used for the vehicle control injections. Each successive injection was into muscle on alternate sides of the breast. Doses are expressed as mg/kg and refer to the salt. Doses shown in the figures are the single dose administered.

RESULTS

Figure 1 shows the drug discrimination curve for pentobarbital and for the effects of pentobarbital on response rate under the drug discrimination schedule (left column), as well as the effects of pentobarbital on responding during both the punished and unpunished components of the multiple schedule (right column). The 1.0 mg/kg dose of pentobarbital resulted in responding almost entirely on the saline key, but with increasing doses there was a graded increase in the percentage of responses on the drug key so that by the 10.0 mg/kg dose responding was confined almost entirely to the drug key. None of these doses produced significant changes in the rate of responding. The dose-response curve for punished responding was very similar to that for drug discrimination with the lowest dose (1.0 mg/kg) producing little effect and increasing doses increasing the rate of responding. In contrast, pentobarbital produced little effect on unpunished responding with only the 3.0 mg/kg dose producing a marginal effect.

Figure 2 shows the effects of diazepam on measures of drug discrimination and on punished and unpunished responding. Diazepam caused a dose-dependent increase in the percentage

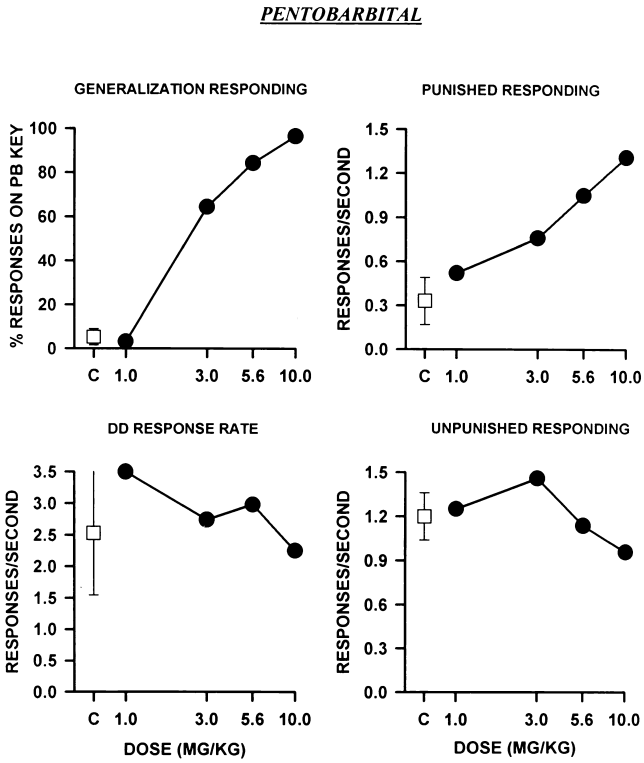

FIG. 1. Effects of pentobarbital on drug discrimination measures (column 1) and rates of punished and unpunished responding (column 2) in pigeons trained to discriminate 5.0 mg/kg pentobarbital from saline. Filled points represent means of single observations in each of four pigeons. Brackets at C represent a ± two standard deviations around the control mean (squares), which is based on 4 observations.

of responses on the drug key, generating a curve very similar to that seen with pentobarbital. Doses above 1.0 mg/kg diazepam decreased the rate of responding under the drug discrimination schedule with the highest dose (5.6 mg/kg) decreasing the rate by about 50%. Diazepam also produced a dose-dependent increase in the rate of punished responding, except that after the 5.6 mg/kg dose the curve had turned over. Diazepam only decreased rates of unpunished responding.

Figure 3 shows the effects of phencyclidine on measures of drug discrimination and on rates of punished and unpunished responding. The 0.3 mg/kg dose of phencyclidine produced responding almost entirely confined to the saline key. Higher doses produced some responding on the drug key, but the percentage of responses on the drug key never exceeded 40%. There is a suggestion that at 1.7 mg/kg phencyclidine the dose-effect curve is beginning to turn over. Phencyclidine only decreased rates of responding under the drug discrimination schedule, beginning at a dose of about 1.0 mg/kg. Phencyclidine produced an inverted U-shaped dose-response curve for punished responding with a "flat peak" from 0.56 to 1.7 mg/kg. A somewhat similar dose-effect curve was generated for unpunished responding, except that rate-decreasing effects were pronounced at the upper end of the dose-effect curve.

Figure 4 shows the effects of buspirone on measures of drug discrimination and on punished and unpunished responding. At doses of 0.1 to 5.6 mg/kg buspirone, responding was confined to the saline key. The highest dose studied (5.6 mg/kg) reduced the rate of drug-discrimination responding.

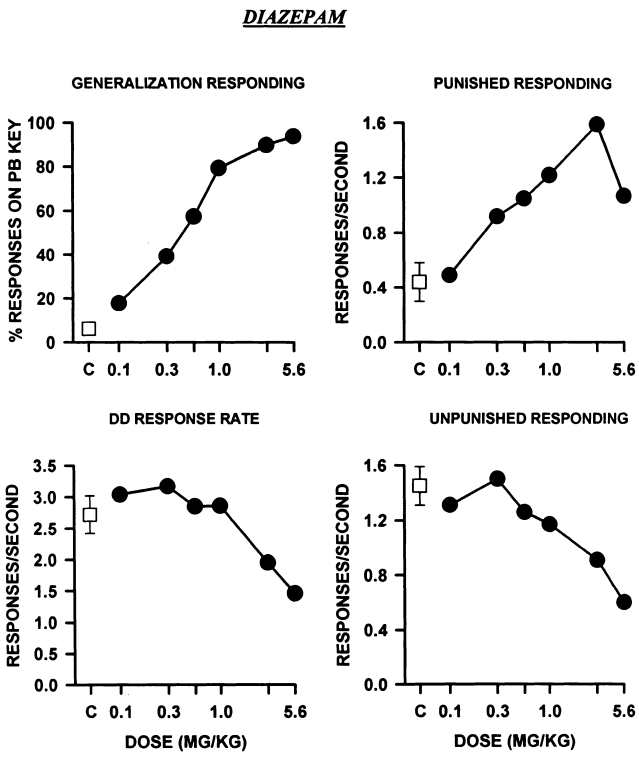

FIG. 2. Effects of diazepam on drug discrimination measures and on punished and unpunished responding in pigeons trained to discriminate 5.0 mg/kg pentobarbital from saline. The control mean is based on 6 observations. Other details as in Fig. 1.

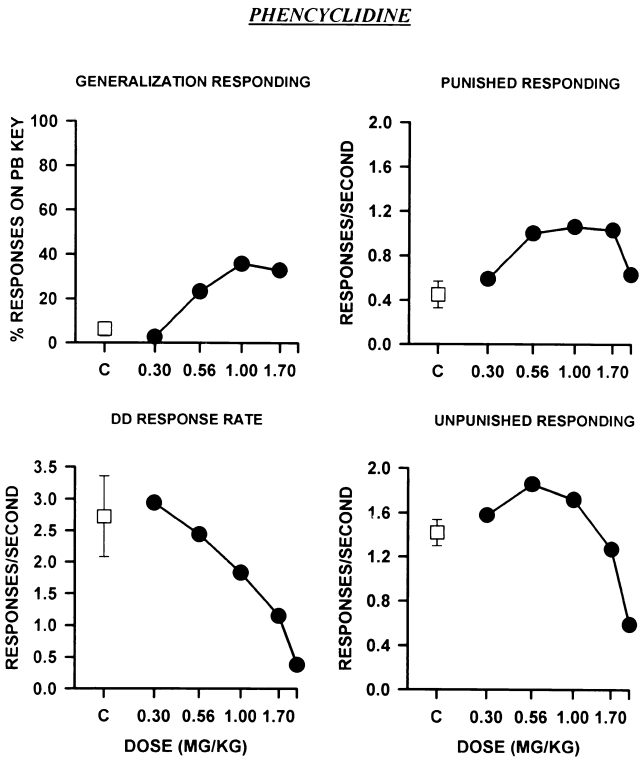

FIG. 3. Effects of phencyclidine on drug discrimination measures and on punished and unpunished responding in pigeons trained to discriminate 5.0 mg/kg pentobarbital from saline. The control mean is based on 5 observations. Other details as in Fig. 1.

Buspirone produced small, but clear increases in punished responding across a range of doses from 0.56 to 1.8 mg/kg, but unpunished responding did not show similar increases in rate. Doses of 3.0 mg/kg and higher decreased rates of unpunished responding.

Figure 5 shows the effects of methamphetamine on measures of drug discrimination responding and on punished and unpunished responding. At doses up to those that suppressed response rate (5.6 mg/kg), methamphetamine did not produce responding on the drug key, nor did methamphetamine increase rates of either punished or unpunished responding. The 5.6 mg/kg dose of methamphetamine decreased the rate of unpunished responding and both the 3.0 and 5.6 mg/kg doses of methamphetamine decreased rates of punished responding.

DISCUSSION

To our knowledge, this is the first experiment studying the effects of drugs as discriminative stimuli and the effects of these same drugs on punished and unpunished responding in the same birds during the same session. This procedure allows the obvious advantage of elimination of intersubject variability in comparisons between the effects of a drug on punished responding and its discriminative stimulus properties, an advantage not obtained in previous studies such as those of McMillan et al. (9). Variations on the procedure should also make it possible to study the relationship of the discriminative stimuli produced by drugs to their effects on a variety of other behaviors.

In pigeons trained to discriminate 5.0 mg/kg pentobarbital from saline, the pentobarbital generalization curve had a shape very similar to the shape of the dose-response curve for the effects of pentobarbital on punished responding. Such data suggest that pentobarbital increases punished responding only at doses that are beginning to be discriminated by the subject. A similar argument has already been advanced by McMillan et al. (9) for the arylcyclohexylamines. The present experiments extends this argument to the barbiturates and perhaps to other drugs as well.

There was complete generalization of the pentobarbital discriminative stimulus to diazepam and the diazepam generalization curve was similar in shape to the pentobarbital generalization curve. The dose-response curve for the effects of diazepam on punished responding was also similar in shape to the diazepam generalization curve over most of the dose range. The dose-response curve for diazepam turned over at the highest dose level for punished responding, but not for drug discrimination; however the turnover of the punishment dose-response curve probably resulted from the rate-decreasing effects of high doses of diazepam as demonstrated from the rate decreases produced by the 3.0 and 5.6 mg/kg doses of diazepam for response rate under both the drug discrimination schedule and the component of the mult VI VI schedule where responses were not punished. Thus with the exception of the effects of the highest dose of diazepam on punished responding, the four dose-effect curves (drug discrimination and punished responding for pentobarbital and diazepam) were very similar to each other.

The close correspondence of these four dose-effect curves suggests the possibility that the discriminative stimuli pro-

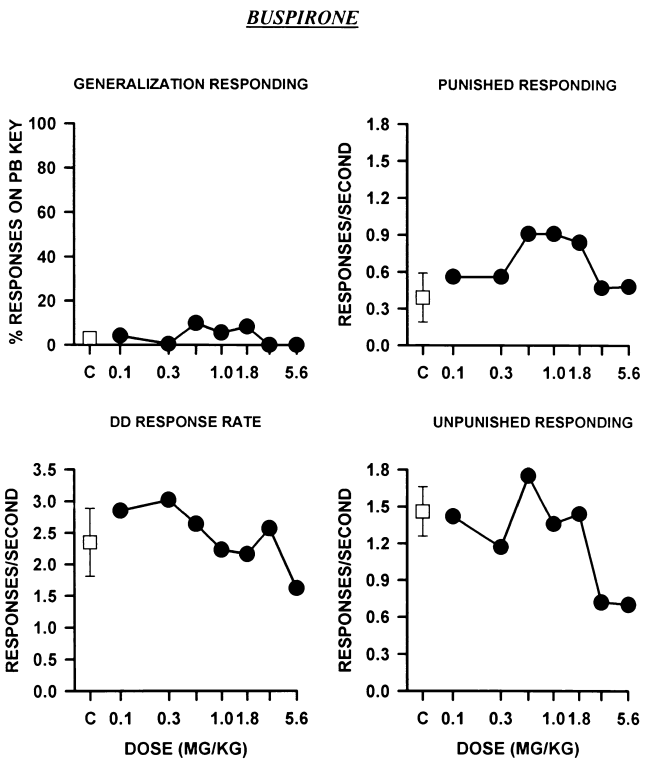

FIG. 4. Effects of buspirone on drug discrimination measures and on punished and unpunished responding in pigeons trained to discriminate 5.0 mg/kg pentobarbital from saline. The control mean is based on 7 observations. Other details as in Fig. 1.

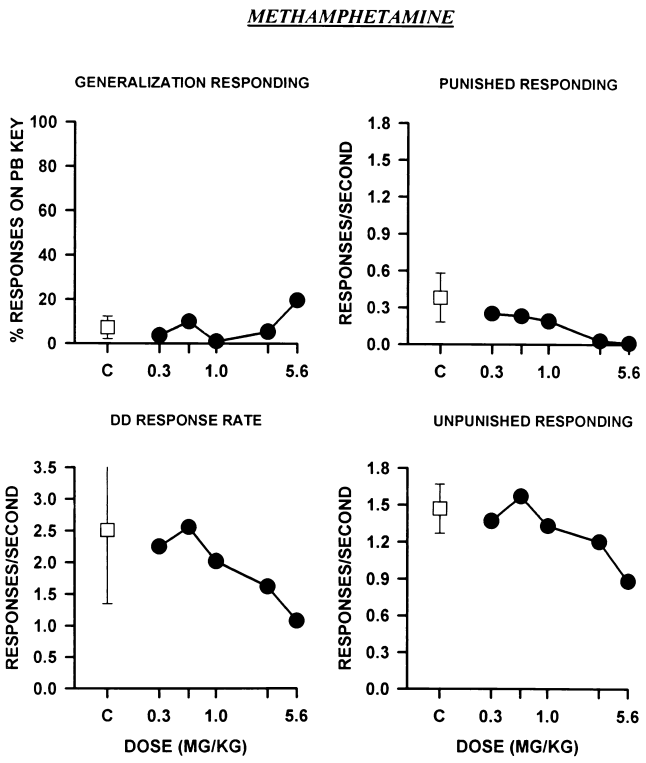

FIG. 5. Effects of methamphetamine on drug discrimination measures and on punished and unpunished responding in pigeons trained to discriminate 5.0 mg/kg pentobarbital from saline. The control mean is based on 5 observations. Other details as in Fig. 1.

duced by these two drugs are closely related to their antipunishment effects. There are several possibilities. First, it could be that there is a common mechanism that underlies the stimulus effects and the antipunishment effects of these drugs, although they are not directly related to each other as to cause and effect. The common mechanism would not be at the receptor level, since it is clear that pentobarbital and diazepam have different molecular mechanisms of action (1); however, benzodiazepine agonists and barbiturate agonists both appear to act on the $GABA_A$ receptor complex, although at different sites (3). Perhaps the increased influx of chloride ions that barbiturates and benzodiazepines produce is related to both their discriminative stimulus effects and their antipunishment activity. A second possibility is that the discriminative stimulus effects of these drugs and their antipunishment activity are more directly related, or even part of the same response. Possibly these drugs produce a state of "decreased anxiety" which contributes to discriminative stimulus properties of the drug and is also the basis for the anti-punishment effects of the drugs.

Some support for this latter speculation comes from the phencyclidine data. Phencyclidine produced at most a partial substitution for the pentobarbital training drug, suggesting that perhaps the phencyclidine stimulus had some common elements with the pentobarbital stimulus, but was not identical to it. Phencyclidine was also much weaker than pentobarbital in increasing punished responding. At the receptor level phencyclidine binds at sites on a receptor complex (15) that is clearly different from the receptor complex at which the barbiturates bind. An interpretation of these data might be that the stimulus effects of phencyclidine only partially resemble those of the barbiturates and therefore the effects of phencyclidine on punished responding are also somewhat different.

Buspirone increased punished responding to about the same extent as pentobarbital and diazepam, as has been reported by others for barbiturates and benzodiazepines (9,14), but there was no generalization from pentobarbital to buspirone, again as reported by others (1,5). These data demonstrate that not all drugs that increase punished responding have similar discriminative stimulus effects, which destroys the thesis that there is any direct relationship between the discriminative stimulus effects and the anti-punishment effects of all drugs that increase punished responding. The data may mean that buspirone and related drugs produce a qualitatively different stimulus state from the benzodiazepines, which may or may not contribute to the anti-punishment effect of buspirone drugs. In this context it would be interesting to know if there are any drugs that substitute for buspirone in the drug discrimination procedure, but do not increase rates of punished responding.

Finally, the effects of methamphetamine were studied merely to include a drug that produced neither an increase in punished responding, nor a pentobarbital-like discriminative stimulus. It fulfilled both criteria in the present experiment. Methamphetamine did not increase unpunished responding in these experiments, as might have been expected. This may have been because the rates of baseline unpunished responding were approximately 1.5 responses/second, which is a control rate of responding above those which are usually increased by the amphetamines (4).

This new procedure holds much promise for relating the stimulus properties of drugs to their other effects on behavior, but the procedure does have some limitations. The position and perhaps the shape of the drug discrimination dose-effect curve, as well as the degree to which generalization occurs to other drugs, depends on the training dose (6). If a different training dose than the 5.0 mg/kg dose of pentobarbital had been used, the drug discrimination and the punishment dose-effect curves might have been less similar. Whether or not similar effects would be obtained with training drugs is also unknown. Clearly, some way of equating training doses in drug discrimination experiments will need to be developed before comparisons can be made across drugs using this procedure

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