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Interaction of the Discriminative Stimulus Effects of Phencyclidine With Those of (+)-N-Allylnormetazocine, Pentobarbital and *d*-Amphetamine[1]

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McMILLAN, D. E. AND W. D. WESSINGER. *Interaction of the discriminative stimulus effects of phencyclidine with those of (+)-N-allylnormetazocine, pentobarbital and* d*-amphetamine*. PHARMACOL BIOCHEM BEHAV **32**(3) 711–715, 1989.—Pigeons trained to discriminate 1.0 mg/kg phencyclidine from saline were used to study the interaction between the stimulus effects of phencyclidine and those of (+)-N-allylnormetazocine [(+) NANM], pentobarbital and *d*-amphetamine using a cumulative-dosing procedure. Both (+) NANM and pentobarbital enhanced the discriminative stimulus effects of phencyclidine. The enhancement of the phencyclidine stimulus by pentobarbital was predicted by adding the effects of the individual drugs, but the enhancement of the phencyclidine stimulus by (+) NANM was sometimes more than would have been expected from adding the effects of the individual drugs. *d*-Amphetamine did not enhance the discriminative stimulus effects of phencyclidine, but neither did it interfere with these effects. Combinations of (+) NANM or pentobarbital with phencyclidine also enhanced the rate-decreasing effects of phencyclidine, but to a lesser extent than they enhanced the discriminative stimulus effects of phencyclidine. *d*-Amphetamine only slightly enhanced the rate-decreasing effects of phencyclidine.

Phencyclidine N-allylnormetazocine Pentobarbital *d*-Amphetamine Discriminative stimulus Drug interactions Pigeons

IT has been well documented that users of psychoactive drugs frequently use more than one such drug at a time. For example, it has been estimated that about half of the time that a person uses an illicit drug such as marijuana, cocaine or a hallucinogen, he or she is also using another illicit drug or alcohol, or both (8). There are a number of possible reasons for the self administration of such drug combinations, at least some of which are related to the interoceptive stimuli produced by the drug combination. Drugs might be combined in an attempt to modulate a punishing component of the stimulus complex produced by one of the drugs, to produce a novel state which does not occur with either drug alone, or to enhance the characteristic stimuli of one or both of the drugs. For example, humans frequently combine tripelennamine and pentazocine to enhance the heroin-like effects of pentazocine. In rats trained to discriminate morphine or N-allylnormetazocine from saline, tripelennamine enhances the morphine stimulus effects of pentazocine, presumably by decreasing the psychotomimetic effects of pentazocine (11).

In the present experiments we used a drug discrimination procedure (4) to study the interactions of phencyclidine (PCP) with other drugs. The drugs chosen for study were pentobarbital, (+)-N-allylnormetazocine [(+) NANM], and *d*-amphetamine. These drugs were chosen for interaction with PCP because the PCP stimulus generalizes completely [(+) NANM], partially (pentobarbital), or not at all (*d*-amphetamine) to these drugs based on previous studies in pigeons from our laboratory (3–6).

METHOD

Subjects

Five White Carneaux pigeons, approximately two years of age at the beginning of these experiments and weighing 480–502 g with free access to food and water, were used in all experiments. The birds were food-deprived to 80% of their free-feeding weights throughout the experiments. All birds were experimentally naive

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at the beginning of the experiments.

Apparatus

The experimental chamber was a standard pigeon test cage equipped with a Gerbrands feeder and three response keys, each of which could be transilluminated with several colors by a 28-V DC key-light assembly containing two 0.04-W bulbs for each key color (Model G7313, Gerbrands Corp., Arlington, MA). The chamber was enclosed inside a Gerbrands Model G7211 sound- and light-attenuating enclosure. The minimum force required to operate the keys was 0.15 N. A relay mounted inside the chamber operated whenever the key contacts were opened on a side key to produce audible feedback for responses. Two 28-V DC houselights illuminated the experimental chamber during the session except during a food cycle. A TRS-80, Model III (Tandy Corp.) microcomputer, and interface (Microcomputer Interface II, MED Associates, Inc., East Fairfield, VT) controlled the schedule and recorded the data.

Procedure

The training of pigeons under this procedure has been discussed in detail previously (4). The schedule in effect at the beginning of these experiments required the pigeon to peck a center key when it was illuminated with a white light. A peck on the center key extinguished it and lighted the two side keys, one with a red light and one with a green light. Five responses on either side key (fixed-ratio 5, or FR 5 component) extinguished both side-key lights, reset the ratios on the side keys to 5, and relighted the center key to reinstate the original condition. Position of the red and green colors on the side keys varied randomly after each center-key response. Food (6-sec access to grain) was presented only after a total of 10 FR 5 components had been completed on the correct side key. Using the terminology of Kelleher (2) for second-order schedules, this schedule is referred to as FR 10 (FR 5). Pecks on the red key were defined as correct and produced food under the FR 10 (FR 5) schedule if 1.0 mg/kg phencyclidine had been administered before the session, and pecks on the green key were defined as correct if saline had been administered before the session. Pecks on the incorrect key did not affect the number of FR 5s required on the correct key for food delivery. On training days, sessions terminated after food presentation had occurred six times, or after 20 min, whichever occurred first. Sessions were conducted Monday through Friday.

During the first 3 or 4 days of the week, the pigeons were given saline, or 1.0 mg/kg PCP, 10 min before the session (the number of days depended on the stability of performance). On Thursdays or Fridays, cumulative dose-effect curves were determined. A bird was injected intramuscularly and placed into the chamber. After 5 min, the session was initiated and it was terminated with the first food delivery. Immediately after food delivery, the bird was removed from the chamber and given a second injection and the process was repeated. Repetitions of the procedure continued until a cumulative dose was reached that disrupted responding such that the bird did not obtain food within 600 sec, or until a predetermined maximum cumulative dose of drug was reached [5.6 mg/kg (+) NANM, 10.0 mg/kg pentobarbital, or 1.7 mg/kg *d*-amphetamine]. All doses shown in the figures are cumulative doses (the sum of all doses given to the bird during that session). Drugs were administered and doses calculated as follows: phencyclidine hydrochloride, sodium pentobarbital, (+)-N-allylnormetazocine hydrochloride or *d*-amphetamine sulfate. Each drug was dissolved in 0.9% saline and administered in a volume of 1.0 ml/kg of body weight.

Drug interactions were determined using a similar cumulative-dosing procedure. First, a "cumulative" saline curve was determined (four or five consecutive saline injections given as described above). During the next cumulative-dosing session, a dose of PCP was given followed by a series of saline injections to establish the time course of stimulus control exerted by each dose of PCP. During the next cumulative-dosing session, a saline injection was given followed by a series of doses of pentobarbital to establish the cumulative dose-effect curve for pentobarbital alone. During the next cumulative-dosing session, the PCP dose was given again, followed by cumulative doses of pentobarbital. During the next few weeks, the cumulative-dosing sessions consisted of cumulative pentobarbital doses with increasingly higher doses of PCP. Upon completion of the study of interactions between PCP and pentobarbital, a similar series of experiments were performed to study the interaction of PCP with (+) NANM and with *d*-amphetamine.

Discrimination data were plotted as the percentage of total responses on the drug-appropriate key (PCP key). An analysis of the percentage of fixed-ratio components completed on the PCP key generated very similar data and are not reported. The average rate of responding on the side keys was also plotted.

RESULTS

The interaction between the discriminative stimulus effects of PCP and those of the other drugs is shown in Fig. 1. As shown by the brackets in the first column of panels in the figure, cumulative saline injections resulted in very few responses occurring on the PCP key. The effects of each dose of PCP (0.1, 0.3, 0.56 and 1.0 mg/kg PCP) combined with saline for a time-course determination is shown by the triangles for the 0.1 mg/kg dose (first column, single determination) and by the shaded portions for increasingly higher doses (mean ± 1 S.D., 6 determinations). The 0.1 mg/kg PCP doses followed by successive saline injections generated responding largely on the saline key (triangles, first column). The shaded areas in the figure show that larger doses of PCP followed by saline (frames left to right) generated increasing amounts of responding on the PCP key.

The top row of Fig. 1 shows the interaction between PCP and pentobarbital. No dose of pentobarbital given after saline (filled points) generated more than 40% of the responses on the PCP key. When pentobarbital was given after PCP (open points), the drug combination produced increased responding on the PCP key compared to that produced by pentobarbital after saline. The greatest increase in PCP-key responding occurred after the 10 mg/kg dose of pentobarbital. A cumulative dose of 17.5 mg/kg pentobarbital after saline eliminated responding, so this cumulative dose was not given after any of the PCP doses.

The second row of Fig. 1 shows the interaction of (+) NANM with PCP. Doses of 0.3 to 3.0 mg/kg (+) NANM produced responding largely confined to the saline key. After the highest dose of (+) NANM (5.6 mg/kg) given after saline, all birds responded entirely on the PCP key (filled points). In this respect (+) NANM differed from pentobarbital which generated only partial responding on the PCP key. Doses of 0.3 to 3.0 mg/kg of (+) NANM increased responding on the PCP key when combined with all doses of PCP (open points) relative to the effects of these same doses of (+) NANM given after saline (filled points). Thus, (+) NANM, like pentobarbital, enhanced the discriminative stimulus properties of PCP.

The bottom row of Fig. 1 shows the interaction between PCP and *d*-amphetamine. *d*-Amphetamine after saline did not produce significant responding on the PCP key. Although there is a suggestion that the 1.7 mg/kg dose of *d*-amphetamine may have slightly increased responding on the PCP key when combined with

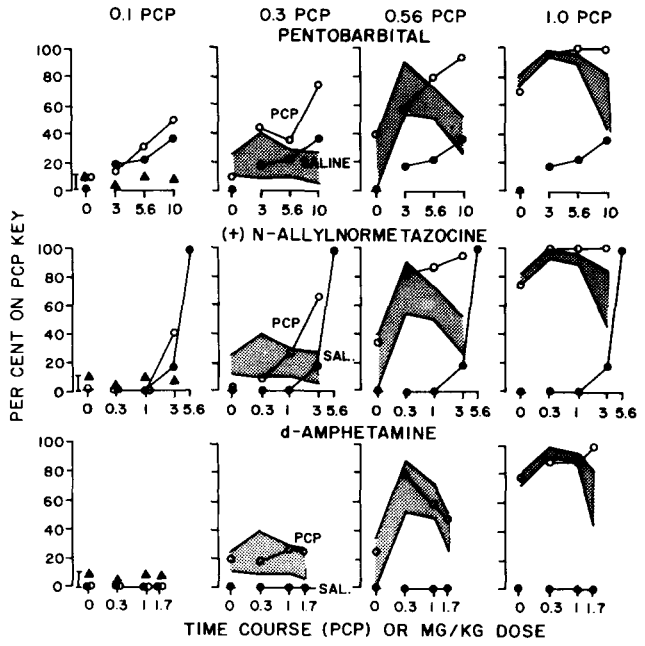

FIG. 1. Interaction of drugs with the discriminative stimulus effects of PCP. Abscissa: mg/kg dose of pentobarbital (top row of panels), (+)-N-allylnormetazocine (middle row of panels) and *d*-amphetamine (bottom row of panels). Ordinate: Percentage of responses on the PCP key. Brackets near the ordinate show ±1 S.D. of means based on six cumulative saline curves (26 observations). The effects of PCP followed by successive saline injections are shown by the triangles for 0.1 mg/kg PCP (left-hand column, single determination) or by the shaded areas for the higher PCP doses (0.3, 0.56 and 1.0 mg/kg PCP), which represent ±1 S.D. around the mean of 6 determinations. Filled points show the effects of saline alone (at 0), or saline followed by cumulative doses of drug. Unfilled points show the effects of PCP followed by saline (at 0) or followed by increasing doses of pentobarbital (top row), (+) NANM (middle row) or *d*-amphetamine (bottom row). The combination of 5.6 mg/kg (+) NANM and 0.56 mg/kg PCP was not studied.

the 1.0 mg/kg dose of PCP, in general, *d*-amphetamine neither increased nor decreased responding on the PCP key when it was combined with PCP.

In an attempt to compare the interactions between PCP and pentobarbital, PCP and (+) NANM, and PCP and *d*-amphetamine, the observed effects of drug combinations were compared with an expected value based on addition of the effects of the individual drugs (Table 1). The expected values were calculated by adding the means of the PCP plus saline observations to the effects of pentobarbital, (+) NANM, or *d*-amphetamine after saline observations (filled points, Fig. 1). If the difference between the expected value and the observed effect of the drug combination was larger than the variability (two standard deviations) around the PCP mean after saline, a greater than effect additive interaction was considered to have occurred. Table 1 shows that greater than effect-additive interactions occurred only with combinations of PCP and (+) NANM.

Figure 2 shows drug interactions between PCP and other drugs for rate of responding on the side keys. During successive saline administrations, the rate of side-key responding averaged about 1.7 responses/sec (brackets in left-hand column of panels). PCP followed by saline injections had minimal effects on rate of responding (filled triangles for 0.1 mg/kg PCP and shaded areas for higher doses of PCP).

TABLE 1

OBSERVED AND EXPECTED (BASED ON AN EFFECT ADDITION MODEL) VALUES FOR PERCENTAGE OF RESPONSES ON PCP KEY FOR DRUG INTERACTIONS

mg/kg Dose		% on PCP Key	
PCP	Pentobarbital	Observed	Expected
0.3	3.0	44.5	37.7
	5.6	36.5	41.0
	10.0	74.5	53.3
0.56	3.0	58.8	90.4
	5.6	80.2	84.2
	10.0	94.0	76.8
	3.0	97.3	100.0
1.0	5.6	100.0	100.0
	10.0	100.0	100.0
	(+) NAMN		
0.3	0.3	8.0	25.4
	1.0	26.6	19.2
	3.0	67.4*	34.1
0.56	0.3	82.6	72.2
	1.0	87.4*	62.4
	3.0	95.2*	57.6
	0.3	100.0	98.0
1.0	1.0	100.0	93.5
	3.0	100.0	82.4
	d-Amphetamine		
0.3	0.3	18.2	26.0
	1.0	26.8	19.4
	1.7	26.5	16.3
1.0	0.3	80.4	72.8
	1.0	59.0	62.6
	1.7	49.5	39.8
3.0	0.3	89.8	98.6
	1.0	90.8	93.7
	1.7	100.0	64.6

*Observed minus expected value was more than twice the standard deviation for the PCP mean (shaded areas in Fig. 1).

Cumulative doses of pentobarbital after saline (filled points, top row, Fig. 2) also had little effect on rate of responding until the 17.5 mg/kg dose eliminated responding. When the pentobarbital dose-effect curve was determined in the presence of increasing doses of PCP (unfilled points), the 10 mg/kg dose of pentobarbital consistently decreased responding.

The second row of Fig. 2 shows the interaction between PCP and (+) NANM for rate of responding. Cumulative doses of (+) NANM after saline (filled points) had little effect, although the 5.6 mg/kg dose of (+) NANM may have produced small response-rate decreases. Only after the 1.0 mg/kg dose of PCP was there convincing evidence that there was interaction between PCP and (+) NANM for rate-decreasing effects.

The bottom row of Fig. 2 shows the interaction between PCP and *d*-amphetamine for rate of responding. There was a suggestion that the combination of 1.7 mg/kg *d*-amphetamine with various doses of PCP produced small rate-decreasing effects not seen with either PCP and saline or *d*-amphetamine and saline.

DISCUSSION

Both pentobarbital and (+) NANM enhanced the discrimina-

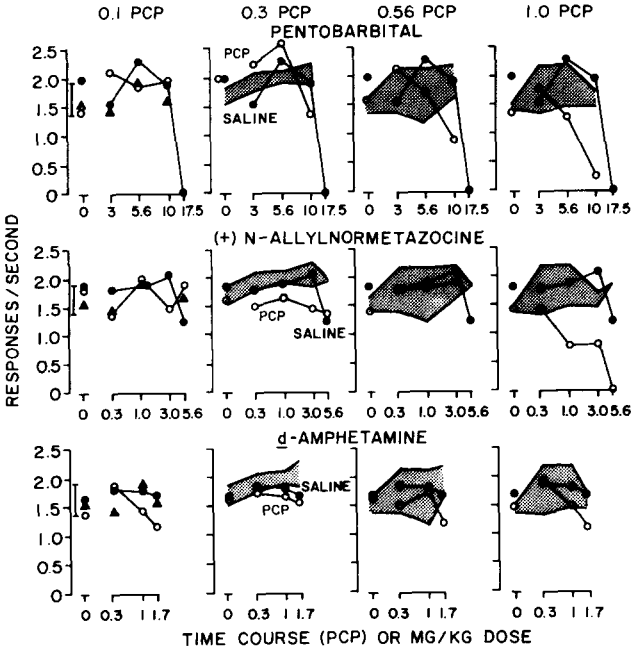

FIG. 2. Interaction of PCP and drugs on rates of responding. Abscissa: mg/kg dose of pentobarbital (top row of panels), (+)-N-allylnormetazocine (middle row of panels) and *d*-amphetamine (bottom row of panels). Ordinate: Rate of responding on the side keys during entire sessions. Brackets near the ordinate show ±1 S.D. of means based on six cumulative saline curves (26 observations). The effects of PCP followed by successive saline injections are shown by the triangles for 0.1 mg/kg PCP (left-hand column, single determination) or by the shaded areas for higher PCP doses, which represent ±1 S.D. around the mean of six determinations. Filled points show the effects of saline alone (at 0) or saline followed by cumulative doses of drug. Unfilled points show the effects of PCP followed by saline (at 0) or followed by increasing doses of pentobarbital (top row), (+) NANM (middle row) or *d*-amphetamine (bottom row). The combination of 5.6 mg/kg (+) NANM and 0.56 mg/kg PCP was not studied.

tive stimulus effects of PCP in pigeons trained to discriminate 1.0 mg/kg PCP from saline. In contrast, *d*-amphetamine did not enhance the discriminative stimulus effects of PCP, nor did *d*-amphetamine interfere with the discriminative stimulus effects of PCP. In previous investigations, there have been reports that combinations of pentobarbital and PCP produced supra-additive effects on complex behavior in pigeons (12), infra-additive effects on variable-interval responding in squirrel monkeys (1) and both infra-additive and supra-additive effects on fixed-interval responding (depending on the dose) in rhesus monkeys (16). Both infra-additive (9) and supra-additive (13) effects of combinations of *d*-amphetamine have been reported. Because both species and schedule have been varied across these studies, there are no generalizations that can be made about interactions of either *d*-amphetamine or pentobarbital with PCP. We do not know of any studies on the interactions between PCP and N-allylnormetazocine using behavioral baselines.

It might be expected that (+) NANM would increase the discriminative stimulus effects of PCP in pigeons. There is complete generalization from PCP to (+) NANM in pigeons trained to discriminate PCP from saline (5), an observation that we replicated in the present study (Fig. 1, row 2, filled points). Furthermore, there is considerable evidence suggesting that PCP and NANM bind to the same receptor in brain (10,17). It therefore seems likely that (+) NANM is producing its enhancement of PCP-like stimulus effects by binding to the same receptor to which PCP binds, although the possibility that (+) NANM extends the duration of the discrimination stimulus properties of PCP by decreasing the rate of metabolism of PCP cannot be completely eliminated. An explanation based on metabolism seems unlikely since the 0.1 and 0.3 mg/kg doses of PCP never generated appreciable responding on the PCP key when given alone, yet these doses of PCP did generate responding on the PCP key when combined with (+) NANM.

With pentobarbital, the case is much less clear. In pigeons trained to discriminate PCP from saline there is usually only partial stimulus generalization from PCP to barbiturates (3), although there is clear evidence from other studies that in at least some birds complete generalization occurs (6,7). To our knowledge, there is no evidence that pentobarbital binds to the same receptors as PCP. Nevertheless, pentobarbital clearly increased the discriminative-stimulus effects of PCP. Again, the possibility that pentobarbital is extending the duration of the PCP stimulus by slowing PCP metabolism cannot be eliminated completely, although this explanation is unlikely since pentobarbital appears to increase the PCP-like stimulus effects of low doses of PCP that generated little responding on the PCP key when given alone.

It was difficult to determine if there were differences between the interaction of PCP with (+) NANM and the interaction of PCP with pentobarbital. The approach of studying the effects of cumulative doses of drugs in the presence of a single dose of PCP resulted in a considerable time saving, but at the expense of preventing a more sophisticated analysis of drug interactions with isobolograms. In at least a preliminary attempt to compare drug interactions, it was assumed that an expected interactive effect could be calculated by adding the effect observed with PCP alone to the effect observed with the cumulative doses of the other drugs. The prediction made by this "effect-addition" model (14) could be compared with the actual data obtained during drug interaction experiments (Table 1). The problem with this approach was to arrive at some estimate of variability against which to estimate whether or not the observed values were significantly different from the expected values. The estimate of variability we used was two times the standard deviation around the mean for each point on the PCP plus saline curves. Thus, the difference between the observed value and the expected value (based on an effect-addition model) would have to be more than twice as large as the standard deviation of the means for PCP redeterminations to be considered to be an interaction that was greater than effect additive. When this calculation was made, in three of nine instances the combinations of PCP and (+) NANM resulted in interactions which were significantly greater than effect additive. In no other case was a significantly greater than effect additive interaction observed. On this basis it is suggested that the interaction of PCP stimulus effects with those of (+) NANM differed from those of PCP with pentobarbital.

It might be argued that in the majority of cases in Table 1, neither PCP nor (+) NANM showed an interaction greater than effect addition. The opportunity to see greater than effect addition, however, is limited by the ceiling placed on the dose-effect curves (no more than 100% of the responses can occur on the PCP key) and the stringent criterion for determining a significantly different interaction than effect additivity.

d-Amphetamine did not increase or decrease the discriminative stimulus effects of PCP. It could be argued that this was because ineffective doses of *d*-amphetamine were used, since cumulative doses of *d*-amphetamine had little effect on either stimulus control or rate of responding. However, in previous studies we have found that increasing the dose by a quarter log step above the 1.7 mg/kg dose used in this study eliminates responding in most birds (6). Furthermore, pigeons have been trained to discriminate 1.0 mg/kg

d-amphetamine from saline (15), so the doses used in the present study (e.g., 1.0 and 1.7 mg/kg) *d*-amphetamine clearly produce discriminative stimulus effects. Thus, *d*-amphetamine produces different discriminative stimulus effects than PCP, since it does not generalize from PCP, yet can itself be established as a discriminative stimulus itself (15). When the discriminative stimulus properties of amphetamine are combined with those of PCP, these different stimulus properties appear to be independent, since their effects were neither additive, nor antagonistic.

Both pentobarbital and (+) NANM enhanced rate-decreasing effects of PCP; however, the effects were largely confined to the upper end of the dose-effect curve for pentobarbital and occurred irregularly with (+) NANM. The interactions between PCP and pentobarbital, or (+) NANM were larger and occurred across a wider range of doses for discriminative stimulus properties than for rate-decreasing effects.

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