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Discriminative Stimulus Properties of Clozapine and Chlorpromazine[1]

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GOAS, J. A. AND J. E. BOSTON, JR. *Discriminative stimulus properties of clozapine and chlorpromazine.* PHARMAC. BIOCHEM. BEHAV. 8(3) 235–241, 1978. – Rats were trained to discriminate pairs of drug states in a two-lever operant paradigm for food reinforcement. One group learned to discriminate clozapine from vehicle, a second group learned to discriminate chlorpromazine from vehicle, and a third group learned to discriminate clozapine from chlorpromazine. The result that the clozapine versus chlorpromazine discrimination was acquired, as well as the results of substitution tests with non-training drugs, suggest that the stimulus properties of the classical neuroleptic chlorpromazine are different from those of clozapine. Substitution tests with clozapine, classical neuroleptics and other psychotherapeutic agents indicate that the stimulus properties of antipsychotics are distinct from other classes of psychotropic agents, and support the hypothesis that clozapine may be a unique antipsychotic. It is suggested that the unique discriminative stimulus produced by clozapine may be related to the differential effect of the drug on the extrapyramidal versus accumbens dopamine system.

Drug discrimination Clozapine Chlorpromazine Discriminative stimulus

PSYCHOACTIVE drugs produce strong, unique physiological stimuli which animals can distinguish from one another and from the nondrug state. Thus, drug-induced interoceptive stimuli are capable of functioning as discrimative stimuli in discrimination learning paradigms [3]. Centrally-acting drugs which produce unique discriminative stimuli include anxiolytic sedatives such as ethanol, barbiturates and benzodiazepines [19]; cholinergics such as nicotine [22]; anticholinergics such as atropine [19]; narcotic analgesics such as morphine [21]; stimulants such as amphetamine [23]; mood-altering compounds such as Δ^9-tetrahydrocannabinol [4] and at least 68 other psychoactive compounds [20].

There have been relatively few reports investigating the discriminative stimulus properties of antipsychotic drugs, and most of these have reported disappointing results. Attempts to train rats to discriminate neuroleptics from vehicle have frequently resulted in either failure to obtain stimulus control with these drugs [15,19] or stimulus control only after extended training [5,12]. Further, the results of substitution tests with drugs other than training drugs have not always selectively separated antipsychotics from non-antipsychotics. For example, although perphenazine has been shown to substitute for chlorpromazine, prochlorperazine did not [25].

More recently, the ability of drugs to block the stimulus properties of the dopamine agonist apomorphine was suggested as a means for identifying the dopamine blocking activity of neuroleptics [11]. Again, although most of the antipsychotic drugs tested blocked the apomorphine stimulus, the commonly-used neuroleptic chlorpromazine did not.

We were interested in investigating in rats the discriminative stimulus properties of antipsychotic drugs administered at non-toxic doses via the oral route, the usual route of administration in man. We chose to study the discriminability of chlorpromazine, a widely used phenothiazine in man, from vehicle, and the discriminability of clozapine, a therapeutically effective dibenzodiazepine antipsychotic with little extrapyramidal syndrome (EPS) liability [17] from vehicle. Also, we were interested in determining whether clozapine and chlorpromazine were discriminable from each other at equieffective doses. If it were possible to generate these discriminations, we were interested in determining which of a number of psychoactive drugs would substitute for these training drugs.

GENERAL METHOD

Animals and Compounds

Male Wistar rats (Royalhart Farms) weighing 200–225 g at the time of arrival were placed individually in cages measuring 25 × 18 × 18 cm for a period of at least one week during which time food (W. F. Fisher Laboratory Rat Diet) and vitamin-enriched water (13.3 ml Poly-Vi-Sol® vitamin syrup/10ℓ water) were always available. After this

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acclimation period the animals were gradually reduced via food deprivation to 80% of their free-feeding body weights and were maintained at those weights for the duration of the experiments.

All compounds were dissolved or suspended (fresh daily) in Universal Vehicle (2% starch, TWEEN-80, polyethylene glycol-400) for oral administration, or in .85% saline for subcutaneous administration. The volume administered was 0.5 ml/100 g body weight. Training sessions were conducted five days per week, one session per day. Daily drug or vehicle doses were given according to the following repeating cycle [10]: A, B, B, A, A; B, A, A, B, B; and so on, Monday through Friday.

Apparatus

Rats were trained and tested in ten Coulbourn Instruments modular operant chambers each equipped with one food pellet module which dispensed 45 mg food pellets (J. P. Noyes Company), two response lever modules (one on either side of the food pellet module) and one houselight module. The operant chambers were enclosed in individual Coulbourn Instruments sound-attenuating chambers and were located in small rooms adjacent to the main laboratory. Control equipment consisted of a PDP-8A computer, a dual-drive floppy-disk unit and a Dec-Writer (all Digital Equipment Corporation), as well as interface and interconnection panels located in the main laboratory.

Shaping and Prediscrimination Training

Rats maintained at 80% of their free-feeding body weights were shaped to lever-press by the method of reinforcing successively closer approximations of the lever-press response. During the shaping process, only one lever was present in the chamber. However, the left-right position of the lever with respect to the food pellet module was alternated during shaping so that each animal learned to lever-press on either side. Once the rat reliably responded on a fixed-ratio 10 (FR 10) schedule of reinforcement, the animal was considered ready to begin prediscrimination training.

Prediscrimination training consisted of ten 20-min sessions of a variable-interval 30-sec (VI 30-sec) schedule of positive reinforcement. During this phase, the training drugs were administered according to the previously described regimen [10] and only the to-be correct lever was present in the chamber, with the to-be incorrect lever removed.

Discrimination Training

The discrimination training phase followed immediately after the prediscrimination training phase. Each session consisted of a mixed (1-min concurrent extinction extinction) (19-min concurrent variable-interval 30-sec extinction) schedule of positive reinforcement with both levers present in the chamber. During the first minute of the session, both levers were correlated with extinction – that is, lever-presses were counted but had no programmed effect. Data generated during this initial reinforcement-free component was intended to measure the degree of stimulus control of the drug state without confounding response selection by presenting reinforcements for correct-lever responses.

The second, 19-min component of the 20-min session was the training and maintenance period of the session during which responses on the correct lever (the lever correlated with the drug state in effect) were reinforced on a VI 30-sec food reinforcement schedule, while responses on the incorrect lever (the lever correlated with the alternate drug state) had no effect. This reinforcement schedule is shown in Table 1.

TABLE 1

RESPONSE CONTINGENCIES IN THE MIXED (1-MIN CONCURRENT EXTINCTION EXTINCTION) (19-MIN CONCURRENT VI 30-SEC EXTINCTION) SCHEDULE OF POSITIVE REINFORCEMENT

	MINUTE 1	MINUTES 2–20
Correct Lever	Responses have no effect	Responses reinforced on VI 30-sec schedule of positive reinforcement
Incorrect Lever	Responses have no effect	Responses have no effect

For half of the animals in each group, the left-hand lever was always correlated with reinforcement during drug state A, while the right-hand lever was correlated with reinforcement during the alternate drug state B. For the other half of the animals in each group, the left-right lever contingencies were the reverse.

The criterion for acquisition of the discrimination for each animal was defined as five consecutive sessions during which first-min (nonreinforced) response accuracy was 80% correct or higher. No substitution tests with drugs other than the training drugs were conducted until this criterion was met.

Substitution Tests

As substitution tests were conducted on Thursdays, it was required that the first-min response accuracy be 80% or higher during the three previous sessions (Monday, Tuesday and Wednesday). If an animal failed to meet this stability criterion during any week, no substitution tests were conducted with that animal.

After administration of a substitution compound, rats were tested for response selection in a one-min session during which no reinforcements were given (1-min concurrent extinction extinction). This substitution test corresponds to the Minute 1 column in Table 1. The test session terminated after the first-min rather than continuing to the 19-min reinforcement period because it was felt that the reinforcing of choice behavior after the animals had been given non-training drugs might alter the specificity of the discrete two-drug discrimination.

EXPERIMENT 1

METHOD

After shaping, thirteen rats began training to discriminate a non-ataxic dose of clozapine (6.0 mg/kg, p.o., one hr absorption [–1 hr]) from vehicle. In those animals

which subsequently met the criteria for discrimination acquisition, a number of other psychoactive agents were tested for their ability to substitute for clozapine. Both chlorpromazine HCl (2.0, 4.0 and 8.0 mg/kg, p.o., −1 hr) and haloperidol (0.25, 0.5 and 1.0 mg/kg, p.o., −1 hr) were tested to determine whether the clozapine stimulus was a general antipsychotic cue. Chlordiazepoxide HCl (6.25 and 12.5 mg/kg, p.o., −1 hr) was tested to determine whether a tranquilizer of the anxiolytic therapeutic class would substitute for clozapine. Atropine sulfate (1.5, 3.0 and 6.0 mg/kg, p.o., −1 hr) was tested to determine whether the clozapine stimulus might be mediated by its reported anticholinergic effects [18]. Finally, chlorpromazine HCl (2.0 mg/kg, p.o., −1 hr) plus benztropine mesylate (1.25, 2.5 and 5.0 mg/kg, s.c. − 1/2 hr) was tested to determine whether a combined antipsychotic-anticholinergic drug effect would mimic the clozapine stimulus.

RESULTS

Of the thirteen rats which began training, eight successfully acquired the clozapine-vehicle discrimination while four died during training and one failed to learn the discrimination after more than 140 sessions.

Figure 1 shows the development of the discrimination during the first-min, non-reinforced period for eight rats. As can be seen in the lower portion of the figure, criterion for vehicle-appropriate responding was met by the fourth block of sessions and was maintained for the duration of the experiment. In contrast, criterion for clozapine-appropriate responding was met during the third block of five sessions and during the seventh block, but did not become stable until the ninth block of sessions after which clozapine-appropriate responding remained above 80% accuracy. Inspection of individual rat data revealed that this slow rate of clozapine control acquisition was not a result of poor performance by one or a few individual rats but was generally representative of the behavior of the entire group during clozapine sessions.

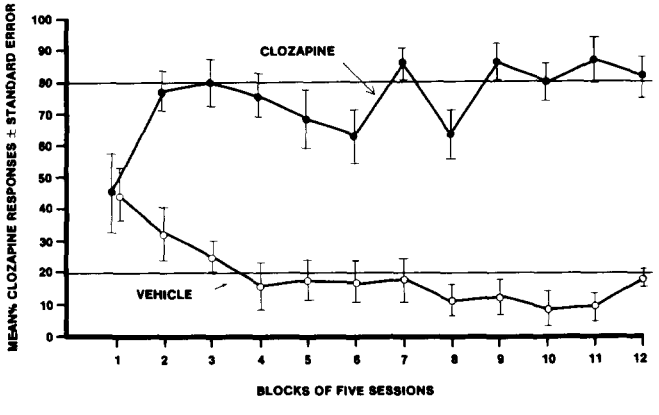

FIG. 1. Mean percent first-minute clozapine responses during clozapine (6.0 mg/kg, p.o.) sessions and vehicle sessions, as a function of blocks of five consecutive sessions. Each data point thus represents an average of 2.5 sessions with each corresponding pair of clozapine and vehicle points representing five sessions. The thin horizontal lines at 80% and 20% denote criteria levels above which and below which clozapine and vehicle responding, respectively, were achieved. N = 8 rats.

The results of substitution tests with non-training drugs tested during the first-min non-reinforced period are shown in Table 2. At the doses tested haloperidol failed to substitute for clozapine, producing more vehicle-appropriate responses than clozapine responses, particularly at the highest dose. Similarly, chlorpromazine did not substitute for clozapine, producing instead a generally even mixture of vehicle- and clozapine-appropriate responses, while chlordiazepoxide cued mostly vehicle-appropriate responses. Like haloperidol, the anticholinergic atropine produced mostly vehicle-appropriate responses, and the combination of chlorpromazine plus benztropine produced the even mixture of vehicle- and clozapine-appropriate responses similar to that produced by chlorpromazine alone.

DISCUSSION

The data from Experiment 1 show that the antipsychotic drug clozapine is discriminable from vehicle, although the stimulus control of the two drug states developed at unequal rates. The vehicle drug state became highly discriminable by about the 16th session of training while the clozapine state did not reach this level of discriminability until about the 46th session (Fig. 1). This asymmetrical acquisition of a drug-vehicle discrimination has been previously reported with other psychoactive agents [7, 14, 16] with one study [16] suggesting that low doses of the training drug may be responsible for the slow development of drug stimulus control in relation to vehicle control. Thus, the relatively low dose of clozapine used in the present experiment may have been responsible for the obtained asymmetry.

Of the drugs tested, none of the non-training compounds substituted for clozapine. As expected, the non-antipsychotic drugs chlordiazepoxide and atropine elicited relatively few clozapine-appropriate responses, consistent with the notion that only therapeutically similar drugs substitute for each other [3]. However, the antipsychotics haloperidol and chlorpromazine as well as the combination of chlorpromazine plus benztropine also failed to substitute for clozapine. Since the latter combination of antipsychotic and anticholinergic drug effects were ineffective in mimicking the clozapine cue, the apparent salience of the clozapine cue might be mediated in part by its lack of striatal dopamine receptor blocking actions, a selective effect not observed after treatment with classical neuroleptic antipsychotics [8].

EXPERIMENT 2

METHOD

Ten rats began training to discriminate a non-ataxic dose of chlorpromazine HCl (2.0 mg/kg, p.o., −1 hr) from vehicle. In those animals which met the criterion for discrimination acquisition, a number of other compounds were tested for their ability to substitute for chlorpromazine. Haloperidol (0.25, 0.5 and 1.0 mg/kg, p.o., −1 hr) was tested in order to determine whether another classical neuroleptic would share similar stimulus properties with chlorpromazine, while clozapine (6.0, 9.0 and 12.0 mg/kg, p.o., −1 hr) was tested to determine whether a low EPS-producing agent would share a common antipsychotic cue with chlorpromazine. As in Experiment 1, chlordiazepoxide HCl (6.25 and 12.5 mg/kg, p.o., −1 hr) was tested to determine whether a tranquilizer of the anxiolytic

TABLE 2

from these laboratories), a possible site of the antipsychotic action of these drugs [1,2]. Thus, differences in the discriminative stimulus properties of the drugs at these doses should reflect differences in side effects such as EPS-liability. In those animals which met criteria for discrimination acquisition, one other compound, haloperidol (0.5, 1.0 and 2.0 mg/kg, p.o., −1 hr) was tested for its ability to substitute for either of the training drugs. Drugs other than antipsychotics were not administered as substitution compounds since, as the rats were forced to choose between two antipsychotic stimuli, interpretation of data from non-antipsychotic substitutions would be of questionable meaning.

RESULTS

All 12 rats which began training successfully acquired the clozapine-chlorpromazine discrimination. Figure 3 shows the development of this discrimination. The lower portion of the figure shows that the criterion for chlorpromazine-appropriate responding was met during the third block of five sessions but did not become stable until the fifth block after which chlorpromazine-appropriate responding remained above 80% accuracy. The upper portion of the figure shows that the criterion for clozapine-appropriate responding was met by the fourth block of sessions and was maintained for the duration of the experiment.

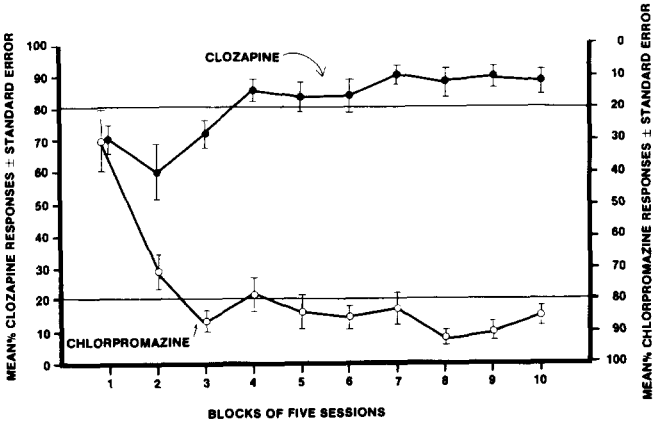

FIG. 3. Mean percent first-minute clozapine responses (left-hand ordinate) and mean percent first-minute chlorpromazine responses (right-hand ordinate) during clozapine (8 mg/kg, p.o.) sessions and chlorpromazine (4.25 mg/kg, p.o.) sessions as a function of blocks of five consecutive sessions. Each data point thus represents a mean of 2.5 sessions with each corresponding pair of clozapine and chlorpromazine points representing five sessions. The thin horizontal lines at 80% and 20% denote criteria levels above which and below which clozapine responding and chlorpromazine responding were achieved. N = 12 rats.

Haloperidol substituted for chlorpromazine at two of the three doses tested. Mean percent chlorpromazine-appropriate responding ± one standard error for each of the doses was: 0.5 mg/kg, $\bar{X}\% = 9.19 \pm 4.0$; 1.0 mg/kg, $\bar{X}\% = 93.3 \pm 4.4$; 2.0 mg/kg, $\bar{X}\% = 73.2 \pm 17.3$ (N = 7 each).

DISCUSSION

The data from Experiment 3 show that the drug-drug discrimination of clozapine from chlorpromazine was readily acquired by rats by about the 21st session. If our assumption that doses which are equivalent in accumbens tyrosine hydroxylase effects are equivalent in therapeutic action, then the discriminable differences between the two drugs may reflect differences in EPS-liability. The obtained transfer data using haloperidol yield preliminary support for this notion. Two of three doses of haloperidol substituted for chlorpromazine and not for clozapine, consistent with reports that both haloperidol and chlorpromazine produce a higher incidence of EPS in man than clozapine which has relatively little EPS-liability [17].

GENERAL DISCUSSION

In contrast to suggestions in some earlier studies [5, 12, 15, 19] the antipsychotic training drugs used in this study seemed to provide interoceptive cues which were distinct from vehicle and from each other. This is supported by the finding that in the present study clozapine and chlorpromazine readily acquired stimulus control over discriminated behavior even at the relatively low doses administered, doses which resulted in minimal changes in overt unconditioned behavior. In other studies the acquisition of the discrimination may have been impaired by the general debilitating effects of the antipsychotics. Clozapine, a unique antipsychotic with minimal EPS-liability [13, 17, 24], was found along with chlorpromazine to be discriminable from vehicle, as well as discriminable from each other.

Within the three discriminations reported here, chlorpromazine versus vehicle (Experiment 2) was the easiest discrimination acquired by the rats followed by the chlorpromazine versus clozapine (Experiment 3) discrimination. The clozapine versus vehicle (Experiment 1) discrimination appeared to be the most difficult to acquire. The degree of training required for a rat to acquire a discrimination is most likely related to both the intensity and the distinctness of the cue provided by the drug. Based upon this assumption, we would conclude that chlorpromazine at doses lower than those of clozapine provides a more discriminable stimulus to the host animal. This could be related to chlorpromazine's greater effects on striatal dopamine [6,9] which in the rat most likely causes the greater degree of neuro-muscular debilitation and which clinically is thought to manifest itself in a higher incidence of EPS side effects.

Some insight into the nature of the drug cue on which the rats base their discriminations can be gained by examination of the substitution studies.

Both chlorpromazine and clozapine will substitute for themselves at various dose levels although the strongest effect is at the dose at which the animals were trained (unpublished observations). In contrast, substitutions between chlorpromazine and clozapine were not consistent. Chlorpromazine did not substitute for clozapine at any dose tested while clozapine did substitute for chlorpromazine at the middle dose. It appears from these results that the clozapine trained rats were cuing on a stimulus which is distinctive from the stimulus used by animals trained to discriminate chlorpromazine from vehicle. While the chlorpromazine animals seemed to be able to recognize some components of clozapine as being similar to chlorpromazine, this recognition was limited to only one of three doses tested. One possible explanation for this result is that when the clozapine animals were given chlorpromazine, the cues produced which are associated with its capacity to cause EPS

TABLE 3

SUMMARY OF SUBSTITUTION TESTS IN RATS TRAINED TO DISCRIMINATE CHLORPROMAZINE (2.0 MG/KG) FROM VEHICLE

Substitution Drug	Dose	Mean Percent Chlorpromazine Responses ± Standard Error	N
haloperidol	0.25 mg/kg, p.o., −1 hr	82.4 ± 17.6	5
	0.5 mg/kg, p.o., −1 hr	100.0 ± 0.0	5
	1.0 mg/kg, p.o., −1 hr	71.4 ± 19.4	5
clozapine	6.0 mg/kg, p.o., −1 hr	43.8 ± 20.5	4
	9.0 mg/kg, p.o., −1 hr	85.4 ± 8.6	4
	12.0 mg/kg, p.o., −1 hr	68.8 ± 18.8	4
chlordiazepoxide HCl	6.25 mg/kg, p.o., −1 hr	56.7 ± 20.3	6
	12.5 mg/kg, p.o., −1 hr	33.3 ± 21.1	6
chlorpromazine HCl 2.0 mg/kg, p.o., −1 hr, plus:			
benztropine mesylate	1.25 mg/kg, s.c., −1/2 hr	89.9 ± 10.1	4
	2.5 mg/kg, s.c., −1/2 hr	77.3 ± 18.9	4
	5.0 mg/kg, s.c., −1/2 hr	56.0 ± 25.9	4

chlorpromazine-appropriate responding was almost at criterion from the first session. Closer inspection of individual rat data revealed that four of the eight rats indeed met the criterion for chlorpromazine-appropriate responding from the first chlorpromazine session. The other four rats responded at about chance levels during these sessions.

Table 3 shows the results of substitution tests with non-training drugs tested during the one-min non-reinforced test session. Haloperidol, at the two lowest doses of 0.25 and 0.5 mg/kg, substituted for chlorpromazine by exceeding the previously described criterion of 80% chlorpromazine-appropriate responding, while the highest dose of 1.0 mg/kg elicited roughly 71% chlorpromazine-appropriate responding. Clozapine, at the lowest dose (6.0 mg/kg) and at the highest dose (12.0 mg/kg), resulted in a somewhat even mixture of chlorpromazine- and vehicle-appropriate responding, while the middle dose (9.0 mg/kg) elicited roughly 85% chlorpromazine-appropriate responding, satisfying the criterion for chlorpromazine substitution. The addition of benztropine to the chlorpromazine stimulus produced a dose-dependent decline in chlorpromazine-appropriate responding, while chlordiazepoxide produced a generally even mixture of chlorpromazine- and vehicle-appropriate responses.

DISCUSSSION

Experiment 2 showed that at the dose used, chlorpromazine is easily discriminable from vehicle. The rats acquired this discrimination by about the 16th session and maintained the discrimination for the duration of the experiment.

The substitution tests with non-training drugs generally yielded results which are consistent with those obtained in Experiment 1. A second classical neuroleptic, haloperidol, clearly substituted for chlorpromazine at all but the highest dose while chlordiazepoxide failed to do so, again supporting the notion that only clinically similar compounds substitute for each other [3]. That one dose of clozapine substituted for chlorpromazine was unexpected, particularly in light of the lack of chlorpromazine substitution for clozapine in Experiment 1. If clozapine and chlorpromazine shared highly similar actions, it would be difficult if not impossible for rats to learn this drug-drug discrimination [19]. Experiment 3 was intended in part to further investigate this question.

EXPERIMENT 3

METHOD

Twelve rats were trained to discriminate clozapine (8.8 mg/kg, p.o., −1 hr) from chlorpromazine (4.25 mg/kg, p.o., −1 hr). These doses were selected because they were shown to produce equal acceleration of tyrosine hydroxylase activity in the rat nucleus accumbens (unpublished data

from these laboratories), a possible site of the antipsychotic action of these drugs [1,2]. Thus, differences in the discriminative stimulus properties of the drugs at these doses should reflect differences in side effects such as EPS-liability. In those animals which met criteria for discrimination acquisition, one other compound, haloperidol (0.5, 1.0 and 2.0 mg/kg, p.o., −1 hr) was tested for its ability to substitute for either of the training drugs. Drugs other than antipsychotics were not administered as substitution compounds since, as the rats were forced to choose between two antipsychotic stimuli, interpretation of data from non-antipsychotic substitutions would be of questionable meaning.

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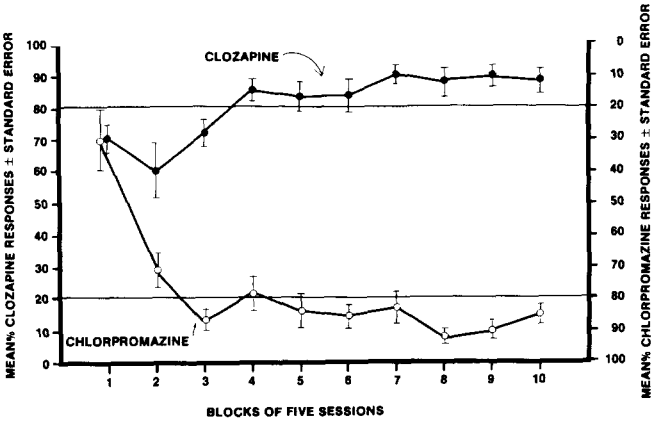

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completely masked any of its cues which are similar to clozapine. The chlorpromazine animals, having extensive experience with chlorpromazine, most likely developed some tolerance to its effects on the extrapyramidal system and as a result detected some of the less overriding cues. These cues may have been similar to some of the clozapine cues and could be related to their common therapeutic activity [13].

To further test the characteristics of the cues provided by these drugs the rats were challenged with an anxiolytic (to test if they were cuing on the anxiolytic properties of the antipsychotics), an anticholinergic (to test if the cue could be related to some anticholinergic properties of the molecule, particularly clozapine) and an anticholinergic plus antipsychotic (in an attempt to distort the chlorpromazine cues if the cue is related to the capacity of the neuroleptics to disrupt the normal functioning of the extrapyramidal system). The results show that the clozapine cue is not related to an anxiolytic property or to an anticholinergic property. Finally, the addition of an anticholinergic to chlorpromazine did not enhance the ability of chlorpromazine to substitute for clozapine (Experiment 1) but did alter the specificity of the chlorpromazine cue in a dose-related manner in the chlorpromazine group (Experiment 2).

A final substitution test using haloperidol, was conducted in the rats trained to discriminate chlorpromazine from clozapine. It was hypothesized that if the difference between the clozapine and chlorpromazine stimuli was related to the differential effects of these drugs on the extrapyramidal system, haloperidol would substitute for chlorpromazine rather than for clozapine. As the results indicated, haloperidol clearly substituted for chlorpromazine, thus lending support to this hypothesis.

Our interpretation of these results is that the antipsychotics which produce EPS effects provide a discriminative stimulus which is distinct from that produced by clozapine. Although there must be common components of these drugs as reflected by their clinical therapeutic similarity and the one instance in this study of clozapine substituting for chlorpromazine, data from the animals trained to discriminate these antipsychotics generally indicated that their stimulus properties are not interchangeable.

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