

Effects of Clozapine on Fixed-Consecutive-Number Responding in Rats: A Comparison to Other Neuroleptic Drugs

MITCHELL PICKER

*Department of Psychology, University of North Carolina at Chapel Hill
Campus Box 3270, Chapel Hill, NC 27599*

Received 10 August 1987

PICKER, M. *Effects of clozapine on fixed-consecutive-number responding in rats: A comparison to other neuroleptic drugs.* PHARMACOL BIOCHEM BEHAV 30(3) 603-612, 1988.—The effects of clozapine and several other neuroleptic drugs were examined in rats responding under fixed-consecutive-number (FCN) schedules with minimum response requirements of 4 and 8. Under these schedules, rats were trained to respond either 8 or more times or 4 or more times on one lever, and then respond once on a second lever. In one component of these schedules, an external discriminative stimulus was presented following the completion of the response requirement on the first lever, whereas no stimulus change was programmed under the other. Under the FCN 8 schedule without the external discriminative stimulus, clozapine produced large dose-dependent decreases in accuracy (percent of reinforced response runs), whereas molindone produced small decreases in accuracy. Neither clozapine or molindone, however, altered accuracy under the FCN 4 without the external discriminative stimulus. Under these same schedules, loxapine, chlorpromazine, haloperidol and thioridazine produced small increases in accuracy at intermediate doses without affecting accuracy at the low and high doses. None of the neuroleptics evaluated produced accuracy-altering effects under the FCN schedules with the external discriminative stimulus. In general, all of these drugs decreased response rates in a dose-dependent fashion. The order of potency for the rate-decreasing effects of these drugs was loxapine > haloperidol > molindone > clozapine = chlorpromazine > thioridazine. Thus, the effects of clozapine on accuracy under the FCN schedules without the external discriminative stimulus differed qualitatively from those of other neuroleptic agents.

Clozapine Conditional discriminations Fixed-consecutive-number Neuroleptics Stimulus control
Rats

ALTHOUGH the dibenzodiazepine clozapine is clinically effective in reducing the symptoms of schizophrenia [15,36], some of its effects are different from those produced by other neuroleptics (antipsychotics). Of considerable importance is the finding that at clinically effective doses clozapine-induced motor side effects are less severe than those commonly associated with neuroleptics of the phenothiazine, butyrophenone and thioxanthene groups [3, 12, 35]. Clozapine also differs from the prototypical neuroleptics in its pharmacological and behavioral profile in nonhumans. Unlike the phenothiazines and butyrophenones, for example, clozapine does not produce catalepsy or muscle rigidity nor does it effectively antagonize apomorphine-induced stereotypies [1,11]. These pharmacological differences are also reflected in clozapine's discriminative stimulus properties. In rats trained to discriminate clozapine from saline, chlorpromazine and haloperidol fail to substitute for the clozapine stimulus. Conversely, in rats trained to discriminate chlorpromazine from saline, haloperidol, but not clozapine, substitutes for the chlorpromazine stimulus [16].

Recent investigations indicate that clozapine differs from

other neuroleptic drugs in its effects on schedule-controlled behavior. For example, clozapine increases low rates of responding under fixed-interval [6, 37, 38] and interresponse-time [9] schedules. Although some notable interspecies differences have been reported [8,42], chlorpromazine and other neuroleptics typically decrease responding under these schedules [6, 9, 32, 37, 43]. In addition, clozapine increases responding suppressed by punishment operations [37] and responding maintained by the presentation of electric shock [4,38], while chlorpromazine and haloperidol only decrease response rates under these schedules [5, 37, 38].

Although the effects of clozapine on schedule-controlled behavior have been examined extensively, there are no reports of its effects in conditional discrimination tasks. In a typical conditional discrimination, reinforcement is dependent upon the presence of at least two stimulus properties of the environment. Thus, in contrast to schedule-controlled performance, conditional discriminations afford the simultaneous evaluation of the effects of drugs on response rate and accuracy of responding. Numerous investigations indicate that there are differences in the effects of various neuro-

leptics when evaluated under conditional discriminations such as the fixed-consecutive-number (FCN) and delayed matching-to-sample procedures (e.g., [21, 31, 40]). In pigeons, the phenothiazine chlorpromazine and the diphenylbutylamine pimozide have been reported to decrease accuracy under FCN [21,40] and delayed matching procedures [25,31] at doses that have no effect on response rates. The butyrophenone haloperidol, in contrast, has been shown to have no effect on accuracy under these tasks even at doses that markedly suppress response rates [21, 25, 31]. Considerably less is known, however, about potential differences in the behavioral effects of neuroleptic compounds in rats responding under conditional discrimination tasks (cf. [14, 17, 26]).

The purpose of the present investigation was to contrast the effects of clozapine with those of several other neuroleptics, including the phenothiazines chlorpromazine and thioridazine, the dihydroindoline molindone, the butyrophenone haloperidol and the tricyclic dibenzoxazepine loxapine on the performance of rats responding under a FCN schedule. Under this schedule, responding a fixed number of times on one lever and then responding once on a second lever is reinforced. Under one variant of this schedule, an external discriminative stimulus is presented following the completion of the response requirement on the first lever, while under the other no external stimulus change is programmed. Thus, the response requirements are the same under both variants of the FCN schedule; however, responding under one variant is controlled by an external discriminative stimulus while responding under the other is controlled by an internal discriminative stimulus. Although these variants of the FCN schedule engender comparable response rates, levels of stimulus control (as reflected in baseline accuracy levels) under the FCN schedule with the added external discriminative stimulus are typically higher than those obtained under the FCN schedule without the added stimulus. Thus, any differential effects observed between the schedules could result from the different baseline levels of stimulus control engendered by each of the schedules and not simply the presence of the added external discriminative stimulus. To examine further the relation between stimulus control and the behavioral actions of neuroleptic compounds, these drugs were evaluated in rats responding under FCN schedules with different minimum response requirements (four and eight). Finally, since repeated exposure to neuroleptics can result in the development of sensitivity or tolerance to their effects, probe tests were conducted during which a selected dose of each drug was redetermined following the completion of each dose-effect determination.

METHOD

Subjects

Eight experimentally-naive male Long-Evans hooded rats, about 4 months old at the start of the experiment, were used. Rats were food deprived to approximately 80% of free-feeding weights (range across rats 300–350 g) and were housed individually with unlimited access to water in a colony maintained on a 12 hr light-dark cycle.

Apparatus

Four plastic and aluminum operant conditioning chambers measuring 23 cm long, 19 cm high, and 20 cm wide were

used. Each chamber was equipped with two 5 cm long response levers located 9 cm from the chamber floor and 1.3 cm from either wall. When operated, a pellet dispenser could deliver a 45 mg food pellet (P. J. Noyes Co., Lancaster, NH) into a pellet trough which was mounted 1 cm above the chamber floor and 6.8 cm from either side wall. Located approximately 15 cm from the floor and 2.5 cm above each lever were two stimulus lights. When illuminated, the lights located above the right lever were red and the lights located above the left lever were white. Two white houselights were mounted on the ceiling 2.5 cm from the rear wall and 8 cm from the either side wall. Each chamber was equipped with an exhaust fan for ventilation and white noise to mask extraneous sounds. Scheduling of experimental events and data collection were accomplished through the use of a TRS model IV microcomputer.

Behavioral Procedure

After preliminary lever press training, four rats were exposed to a fixed-consecutive-number schedule with an external discriminative stimulus (FCN SD). During the initial training sessions, two red stimulus lights located above the right lever (work lever) were illuminated, and a single response on the work lever turned off the red stimulus lights and turned on the white stimulus lights located above the left lever (reinforcement lever). A subsequent response on the reinforcement lever produced a food pellet, turned off the left lever lights, and illuminated the right lever lights. Although recorded, multiple responses on the reinforcement lever had no scheduled consequences. Over the next few sessions the minimum number of responses on the work lever before a response on the reinforcement lever was reinforced was gradually increased to eight. Under this schedule (FCN 8-SD) food was delivered if the rat responded eight or more times on the work lever and then responded on the reinforcement lever. Responding less than eight times on the work lever and then responding on the reinforcement lever reset the response requirement but had no effect on which stimulus lights were illuminated. The houselight remained darkened whenever the FCN 8-SD schedule was in effect. Under these conditions, sessions terminated after 50 reinforcers or 30 min, whichever came first.

When the percentage of reinforced response runs for individual rats showed no visually evident trend, rats were exposed to a multiple FCN schedule. Each session started with the FCN 8-SD, as described above, followed by the FCN 8. The contingencies under the FCN 8 were identical to those under the FCN 8-SD with the exception that when the FCN 8 was in effect the houselight was illuminated, the lever lights were darkened, and no stimulus change was associated with the completion of the minimum response requirement on the work lever. During the initial training sessions, the minimum response requirement on the work lever was gradually increased from 1 to 8. Each component of the multiple schedule was in effect for 5 min or until eight reinforcers were earned. If eight reinforcers were earned before the end of the 5 min component, all lights were darkened until the start of the next component. Each session started with the FCN 8-SD, followed by the FCN 8, and alternated thereafter until three components of each variant of the FCN schedule was completed. Experimental sessions were conducted 5 days per week, at about the same time each day.

For a second group of four rats the minimum response requirement on the work lever was four; that is, a multiple

FCN 4-SD/FCN 4 schedule was arranged. Otherwise, all experimental contingencies for this group of rats were identical to those previously described under the FCN 8-SD/FCN 8 schedule.

Pharmacological Procedure

After 45 sessions of exposure to the multiple FCN schedules described above, dose-effect curves were determined for molindone HCl (Dupont, Wilmington, DE) clozapine (Sandoz, E. Hanover, NJ), thioridazine HCl (Sandoz, E. Hanover, NJ) haloperidol (McNeil, Spring House, PA) loxapine HCl (Lederle, Carolina) (Puerto Rico) and chlorpromazine HCl (Smith Kline and French, Philadelphia, PA) in that order. At least six doses of each drug were given once, in an irregular order, that varied across rats. At the completion of each dose-effect determination, probes were conducted during which a selected dose of each drug, previously determined to decrease response rates by at least 50% under the FCN 8-SD/FCN 8 schedule, was redetermined. Sequences of probe tests were followed and preceded by a minimum of 7 drug free days. Probe testing with molindone HCl, clozapine, thioridazine HCl, haloperidol and loxapine HCl were conducted on 5, 4, 3, 2, and 1 occasions, respectively. During each dose-effect determination and redetermination of selected doses (probe tests), drugs were administered on Tuesday and Friday, whereas distilled water was injected on Thursday with the data obtained during these sessions serving as the nondrug control data.

All drugs and vehicle control were administered IP 30 min prior to the session at an injection volume of 1.0 ml/kg. Molindone HCl, thioridazine HCl and chlorpromazine HCl were dissolved in distilled water. Clozapine and haloperidol base were dissolved in a small amount of 1 N acetic acid and diluted further with distilled water. Solutions of loxapine HCl were obtained by diluting the commercial injectable preparation with a solution consisting of propylene glycol and distilled water. Doses of clozapine and haloperidol are expressed as a base and molindone HCl, thioridazine HCl, loxapine HCl and chlorpromazine HCl as salts.

Data Analysis

The percent of reinforced runs, overall rates of responding, and frequency distributions of run lengths were recorded during each of the FCN schedules. The percent of reinforced runs reflect the proportion of response runs during which a rat completed the minimum response requirement on the work lever (4 or 8) and then responded once on the reinforcement lever. Responding less than the minimum response requirement on the work lever and then responding once on the reinforcement lever was recorded as an error. Conditional probability functions were computed from run length distributions to determine possible drug-induced changes in response patterning. These functions reflect the conditional probability of switching to the reinforcement lever after completing individual response runs of any given length. Percent of reinforced runs and response rates were subjected to an analysis of variance (ANOVA) for each of the four FCN schedules. Comparison of the dose level for each drug was then compared with the control values using a Tukey pairwise comparison test. The *p*-values associated with these comparisons are stated throughout the results section.

TABLE 1

MEAN CONTROL VALUES FOR PERCENT OF REINFORCED RUNS AND RESPONSE RATES FOR INDIVIDUAL RATS UNDER THE MULTIPLE FCN 8-SD/FCN 8 AND FCN 4-SD/FCN 4 SCHEDULES

Rat (No.)	% FCN 8-SD		% FCN 8	
	Reinforced Runs	Responses/ Sec	Reinforced Runs	Responses/ Sec
1	87 (2.9)*	1.11 (0.04)	70 (5.0)	1.30 (0.05)
2	95 (0.4)	1.03 (0.04)	67 (2.9)	1.23 (0.05)
3	97 (1.2)	1.21 (0.04)	84 (3.7)	1.29 (0.07)
4	99 (0.7)	1.01 (0.04)	86 (1.8)	0.97 (0.03)
	% FCN 4-SD		% FCN 4	
5	98 (1.4)	0.75 (0.04)	63 (3.3)	0.84 (0.04)
6	98 (0.6)	0.79 (0.02)	78 (2.9)	0.77 (0.03)
7	100 (0.2)	0.83 (0.02)	88 (1.9)	0.94 (0.02)
8	99 (0.6)	0.84 (0.04)	73 (3.8)	1.00 (0.04)

*Data are based on the mean control values for individual rats during all dose-effect determinations. Values in parentheses indicate the standard error of the mean. All rats earned the maximum number of 24 reinforcers in each variant of the FCN schedules.

RESULTS

The percent of reinforced runs (accuracy) and the shape of the conditional probability functions under each variant of the FCN schedules were similar to those reported previously [28,33]. Data obtained during baseline and vehicle control sessions indicated that the FCN schedules with the added external discriminative stimulus engendered higher accuracy levels than the FCN schedules without the added stimulus, $F(3,92)=44.2$, $p<0.01$ (see Table 1). When a discriminative stimulus was presented following the completion of the response requirement on the work lever mean accuracy levels were 95% and 99% under the FCN 4-SD and FCN 8-SD, respectively; when no discriminative stimulus was presented mean accuracy levels were 76% and 77% under the FCN 4 and FCN 8, respectively. Although no differences in accuracy levels were obtained between the schedules with the different minimum response requirements, there were differences in the shape of the conditional probability functions (see control performance in Fig. 3). For example, the slope of the conditional probability functions under the FCN 4 was considerably steeper than that obtained under the FCN 8, while similar slopes were obtained under the FCN 4-SD and FCN 8-SD. In addition, the peak of the functions under the FCN 4-SD and FCN 4 occurred at runs lengths shorter than those under the FCN 8-SD and FCN 8. Differences between the schedules with the different minimum response requirements were also apparent in response rates, $F(3,92)=48.5$, $p<0.01$, with the FCN 4-SD and FCN 4 engendering response rates that were consistently lower than those obtained under the FCN 8-SD and FCN 8 $p<0.01$; mean response rates under the FCN 4 and FCN 4-SD were 0.80 and 0.89 responses/sec, respectively, and 1.09 and 1.20 responses/sec under the FCN 8 and FCN 8-SD, respectively. Across the course of the experiment, accuracy levels and response rates not change appreciably under either of the FCN schedules.

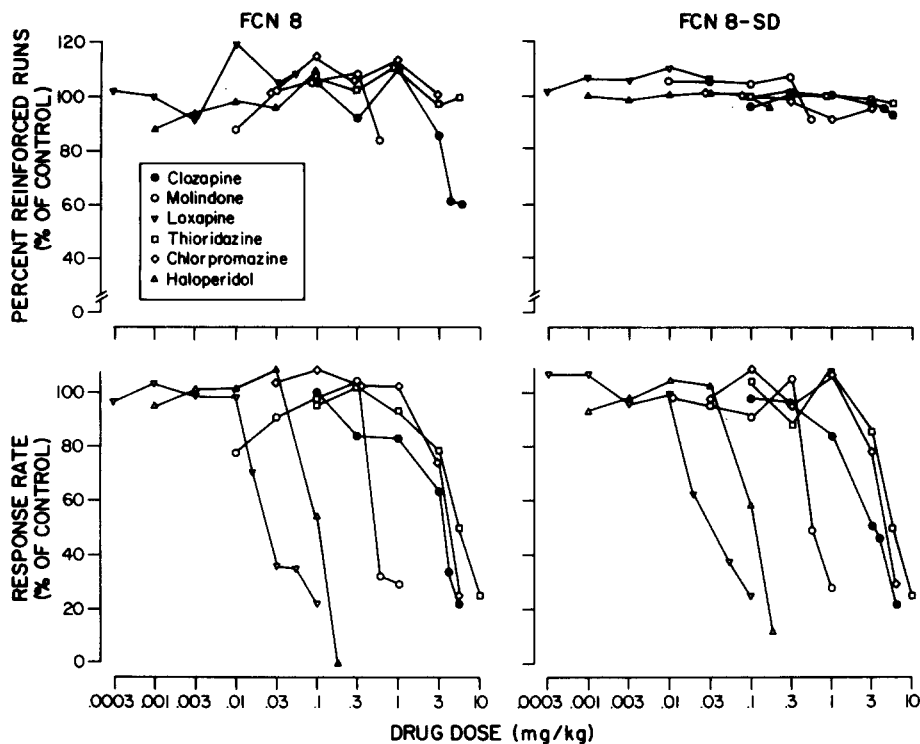


FIG. 1. Effects of clozapine, molindone, loxapine, thioridazine, chlorpromazine and haloperidol on the percent of reinforced runs and response rates in four rats responding under the multiple FCN 8-SD/FCN 8 schedule. For ease of comparison, drug data represent mean group performances expressed as the percent of individual control performances during all control sessions prior to drug administration. Under each variant of the FCN schedule, drug data for percent of reinforced runs were included only when an individual rat earned eight or more reinforcers during the session; data are not displayed for percent of reinforced runs when only one rat met this requirement.

Figure 1 shows the effects of clozapine, molindone, loxapine, thioridazine, chlorpromazine and haloperidol on the percent of reinforced runs and response rates under the FCN 8-SD and FCN 8. Under the FCN 8, clozapine produced large dose-dependent decreases in accuracy. Relative to control values, the 3.0, 4.2 and 5.6 mg/kg doses of clozapine reduced accuracy by 15%, 33% and 40%, respectively. Molindone decreased accuracy by 18% at the 0.56 mg/kg dose, but had no consistent effect at low and intermediate doses. Loxapine, thioridazine and chlorpromazine produced small increases in accuracy that were above the range of control values at intermediate doses under this schedule, but had no consistent effect at the low and high doses. Haloperidol failed to effect accuracy levels across the dose range evaluated. Table 2 shows the maximum increases and decreases in percent of reinforced runs for each of the drugs evaluated under the FCN 8 schedule; for ease of comparison, data are expressed as the mean percent of change from individual control performances. Under the FCN 8 schedule significant ($p < 0.01$) increases in accuracy were obtained for loxapine (0.001 mg/kg) and chlorpromazine (0.1 mg/kg), whereas decreases in accuracy were obtained for molindone (0.56 mg/kg) and clozapine (3.0, 4.2 and 5.6 mg/kg). Even at doses that substantially suppressed mean overall response rates, haloperidol, loxapine and chlorpromazine failed to decrease accuracy. In most instances, doses that reduced response rates for individual rats under

the FCN 8 had no effect on accuracy. Under the FCN 8-SD, none of the neuroleptics evaluated altered accuracy levels, $F(33,102)=1.48$, $p > 0.01$. These drugs did, however, decrease mean response rates in a dose-dependent fashion under both variants of the FCN schedule. For individual rats, dose-response curves for molindone, thioridazine, haloperidol, loxapine and chlorpromazine were typically steep; that is, for individual rats the dose that eliminated responding was typically $1/4$ - $1/2$ log-unit larger than a dose that had no effect on responding.

Figure 2 shows the effects of clozapine, molindone, loxapine, thioridazine, chlorpromazine and haloperidol on the percent of reinforced runs and response rates under the FCN 4-SD and FCN 4. Under the FCN 4, clozapine and molindone had no effect on accuracy across the dose range evaluated. Chlorpromazine, loxapine, thioridazine and haloperidol typically increased accuracy above the range of control values at intermediate doses under the FCN 4 without consistently affecting accuracy at low and high doses. These accuracy-increasing effects were significant ($p < 0.01$) for haloperidol (0.03 mg/kg), loxapine (0.003, 0.01, 0.03 mg/kg) and chlorpromazine (1.0 mg/kg), but not for thioridazine. In addition, no accuracy-altering effects for any of these drugs were observed under the FCN 4-SD, $F(34,105)=0.71$, $p > 0.01$. With the exception of thioridazine, which increased response rates at low doses under the FCN 4, these drugs produced dose-dependent decreases in response rates under

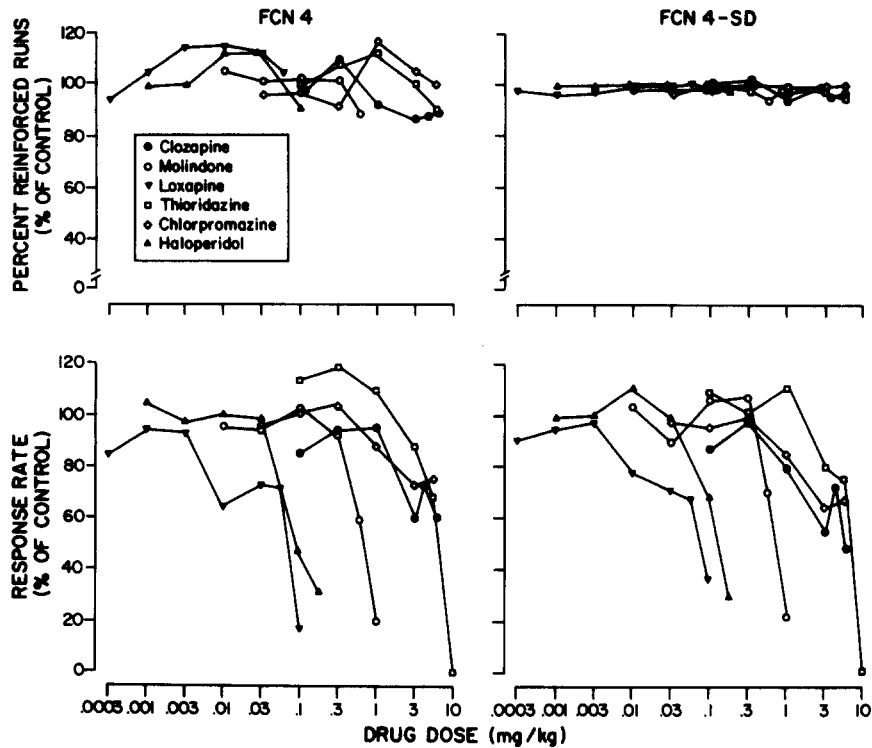


FIG. 2. Effects of clozapine, molindone, loxapine, thioridazine, chlorpromazine and haloperidol on the percent of reinforced runs and response rates in four rats responding under the multiple FCN 4-SD/FCN 4 schedule. Details are as described in Fig. 1.

TABLE 2
 MAXIMUM PERCENT INCREASES AND DECREASES IN PERCENT OF REINFORCED RUNS
 IN RATS RESPONDING UNDER THE FCN SCHEDULES WITHOUT THE
 EXTERNAL DISCRIMINATIVE STIMULUS

Drug	Maximum Increases in % Reinforced Runs† (dose)		Maximum Decreases in % Reinforced Runs† (dose)	
	FCN 8	FCN 4	FCN 8	FCN 4
Molindone	8 (0.3)	6 (0.01)	18 (0.56)*	13 (0.56)
Clozapine	9 (1.0)	7 (0.3)	40 (5.6)*	13 (4.2)
Thioridazine	10 (1.0)	13 (1.0)	4 (3.0)	12 (5.6)
Haloperidol	7 (0.03)	14 (0.03)*	12 (0.001)	10 (0.1)
Loxapine	19 (0.001)*	15 (0.003, 0.01)*	7 (0.003)	6 (0.0003)
Chlorpromazine	15 (0.1)*	18 (1.0)*	1 (3.0)	8 (0.3)

*Tukey pairwise comparisons, significant at $p < 0.01$.

†Data were included only when an individual rat earned 8 or more reinforcers under the FCN 8 or FCN 4 schedules. All data are expressed as the mean percent of change from individual control performances.

Note: Doses are expressed as mg/kg; for loxapine increases of 15% occurred at two doses.

both FCN schedules. In general, for individual rats responding under the FCN 4-SD and FCN 4 dose-response curves were more shallow than those obtained under the FCN 8-SD and FCN 8; that is, low doses had no effect on responding, intermediate doses produced a moderate reduction in re-

sponding and high doses produced a substantial reduction in responding.

Figures 3, 4 and 5 show the effects of clozapine and chlorpromazine, molindone and loxapine, and haloperidol and thioridazine, respectively, on the conditional probab-