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Effects of Clozapine, Chlorpromazine and Diazepam Upon Adjunctive and Schedule Controlled Behaviors

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CANON, J. G. AND A. S. LIPPA. *Effects of clozapine, chlorpromazine and diazepam upon adjunctive and schedule controlled behaviors.* PHARMAC. BIOCHEM. BEHAV. 6(5) 581–587, 1977. – Two twelve-animal groups of rats were trained to press a lever for food reinforcement under either a fixed ratio 20 (FR 20) or a fixed interval 2 min (FI 2 min) schedule. During the FI 2 min schedule a measure of adjunctive behavior (i.e., drinking) was taken. Each group was then administered various doses of chlorpromazine (2.5, 5.0, 10.0 mg/kg, P.O.), clozapine (2.5, 5.0, 10.0 mg/kg, P.O.) or diazepam (5.0, 10.0, 15.0 mg/kg, P.O.) in a random order. All three drugs reliably reduced FR 20 response rates in a dose dependent manner, but chlorpromazine and clozapine were more potent in this regard. Chlorpromazine reduced FI 2 min response rates especially in the terminal portions of the fixed intervals while diazepam generally elevated rates primarily in the mid-portion of the interval. Clozapine produced a less defined effect on overall responding. All three drugs affected index of curvature. Only chlorpromazine was able to reliably reduce occurrence of adjunctive behavior and reinforcements.

Fixed ratio schedule Fixed interval schedule Adjunctive behavior Chlorpromazine Clozapine Diazepam

THE CLASSICAL neuroleptic drugs, such as those from the phenothiazine series, have proven to be extremely useful in the treatment of psychotic disorders. Unfortunately, these agents also produce extrapyramidal symptoms which often limit the use of these drugs in a clinical setting. It is not surprising, therefore, that considerable interest has recently been aroused over a new drug, clozapine, which appears to possess antipsychotic properties without producing significant extrapyramidal side effects [1, 8, 9]. Since clozapine appears to represent a major departure from other neuroleptic drugs, it is important to devise experimental procedures that can be used in laboratory animals to differentiate clozapine's pharmacological activity from other neuroleptic drugs. The introduction of such procedures would allow investigators to screen new compounds for their ability to mimic clozapine's psychopharmacological profile, thus aiding in the development of new antipsychotic drugs with less side-effect liability.

To this end, the present report summarizes the effects of clozapine upon behavior that is controlled by two distinct schedules of reinforcement, FI 2 min and FR 20. By comparing clozapine's effects with those of a standard neuroleptic, chlorpromazine, and a typical anxiolytic, diazepam, an attempt was made to delineate specific behavioral profiles for each type of drug. In addition to monitoring the lever-press rates normally generated during these schedules of reinforcement, a measure of adjunctive behavior (i.e., drinking) was also studied. The use of adjunctive behaviors in assessing drug effects has only recently been introduced and is thus a relatively unexplored phenomenon that might be useful in constructing pharmacological profiles. Adjunctive behavior is a term which has been applied to describe those characteristic, reproducible patterns of behavior that occur in response to the presentation of environmental stimuli that are not dependent upon the occurrence of the adjunctive behavior. Thus, for example, a rat will consistently drink large amounts of water after the ingestion of a food pellet that has been delivered on some type of intermittent schedule that has a relatively low reinforcement density [10]. Thus, adjunctive behavior is unlike schedule controlled eating behavior in that the emission of the drinking response continues in the absence of any consequences other than relieving thirst. The present report makes use of both schedule controlled and adjunctive drinking behavior to characterize the psychopharmacological profile of clozapine, chlorpromazine and diazepam.

METHOD

Animals

The animals for these experiments consisted of 24 male

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Wistar albino rats obtained from Royalhart Farms, Goshen, NY. These animals weighed approximately 250 g when they were received from the supplier. After the animals reached 300 g, body weights were stabilized through restricted feeding. In general, individual animals received approximately 10 g of supplemental feed each day after experimental sessions were completed. Animals had ad lib access to water in their home cages.

Apparatus

The testing chambers consisted of four Gerbrands (Model C) small animal environments from Ralph Gerbrands Co., Arlington, MA. These chambers measured 9 1/2 × 7 1/2 × 8 1/2 in and were contained within four separate sound-attenuated cubicles. A BRS rat lever (Model 121-05) obtained from BRS/LVE Electronics, Beltsville, MD, was mounted 3 1/4 in above the grid floor in the center of the intelligence panel. This lever required a downward pressure of 15 g to activate the spring-loaded micro-switch. A Davis feeder (Model PD-104) from Davis Scientific Equipment Company, Studio City, CA was mounted behind the intelligence panel. It was used to automatically deliver the 0.045 g Noyes food pellets to the food magazine which was located in the left-hand lower portion of the intelligence panel. The house light was powered by 12 VDC and was mounted above the Gerbrands chamber. A 100 ml Richter tube was also attached to the intelligence panel so that the spout was just below and to the right of the food magazine. This tube allowed animals free access to water during the fixed interval 2 min schedule, but was removed during the FR 20 schedule sessions. White noise (80 db) was presented from a speaker located outside the testing chambers (i.e., on one wall of the experimental room) and was used to mask any extraneous noise. Individual fans provided fresh air to each animal chamber throughout all testing sessions. A PDP-8 computer (Digital Equipment Corporation) was located in an adjacent room. It was used to program experimental events and to record behavioral data.

Procedure

Animals were randomly assigned to one of two 12 animal groups (i.e., FR 20 or FI 2 min) and totally deprived of food for 24 hr before lever-press training commenced. All animals were trained to press the response lever to obtain a Noyes food pellet. After this response had been acquired each animal was subjected to two consecutive daily 20 min sessions under a continuous reinforcement schedule (CRF) The FR 20 group was then switched to a fixed ratio 5 schedule for four 1/2 hr daily sessions followed by twenty-four 1/2 hr sessions under a fixed ratio 20 schedule of reinforcement. After this period animals in the FR 20 group demonstrated stable response patterns (i.e., ± 10% variation of 3-day mean response rate) and thus entered the drug portion of the study. After CRF training the FI 2 min animals were subjected to a fixed interval 15 sec schedule for two consecutive 1 hr sessions followed by three 1 hr sessions under a FI 1 min schedule. These animals then received eleven sessions of training under a FI 2 min schedule after which they demonstrated response stability and thus entered the drug portion of the study.

The drugs administered in the present experiment consisted of clozapine (2.5, 5.0, 20.0 mg/kg, base form), chlorpromazine hydrochloride (2.5, 5.0, 10 mg/kg) and diazepam hydrochloride (5.0, 10.0, 15.0 mg/kg). All drugs were administered in a 2% starch vehicle with 1 drop of Tween 80 and 0.5 ml of polyethylene glycol per 10 ml of solution. Clozapine would not dissolved in the starch vehicle and thus 0.1 N HCl was added to the vehicle until the compound dissolved. Control solutions of the vehicle administered prior to clozapine dosing also contained equal amounts of 0.1 N HCl. All drug and control solutions were administered orally (P.O.) one hr before behavioral testing commenced.

The drug portion of the study took place over a seven week period. Each experimental week consisted of five days (i.e., Monday – Friday). Animals were tested the same time each day for a period of either 1/2 hr (FR 20 group) or 1 hr (FI 2 min group). Control solutions were administered on Wednesdays followed by drug dosing on Thursdays. Thus, animals received drug solutions only on one day per week. No solutions were administered on the three remaining days of each week. The nine drug dose combinations were randomly distributed among the two 12 animal groups so that each animal never received the same dose of a drug more than once. Since the drug portion of the study occurred over a seven week period, however, none of the animal's received all nine drug dosages.

RESULTS

Figure 1 presents the mean number of reinforcements (i.e., Noyes pellets) received by the FR 20 animals under various doses of clozapine, chlorpromazine and diazepam. As the data in this figure indicate all three compounds produced significant dose related decrements in the number of reinforcements obtained by this group of animals. Since the number of food pellets are directly proportional to the number of lever-press responses emitted by the animals (1:20) the data in Fig. 1 demonstrate that these drugs dramatically reduce the response rates of animals under the control of an FR 20 schedule. The two antipsychotic drugs, however, appear to be more potent in reducing response rate than was diazepam.

Figure 2 presents the mean response and reinforcement rates for those animals subjected to the FI 2 min schedule under various drug and control conditions. Unlike the FR 20 data, the results summarized in Fig. 2 indicate that each compound had differential effects on response rate. Chlorpromazine produced dose-related decrements in overall response rate. Diazepam produced increments at all doses but only the increment at the low dose was significant ($p<0.05$). This lack of dose response effect appears to be related to the sedative and ataxic producing properties of diazepam as the dose is increased.

Clozapine, on the other hand, produced an elevation in mean response rate at 5.0 mg/kg and decreased the rate at 20.0 mg/kg. These changes were not significant ($p<0.05$), however, the indexes of curvature [12] presented in Table 1 show there was a significant change ($p<0.05$) at 5.0 mg/kg indicating less curvature. At the 10 mg/kg of clozapine two rats showed marked depression of overt locomotor activity and operant response behavior as did rats on the high dose of chlorpromazine suggesting a neuroleptic-like effect. The index of curvature was also altered for both diazepam and chlorpromazine (Table 1). All of these changes reflect a loss of the scallop effect which is characteristic of FI response patterns. The lower

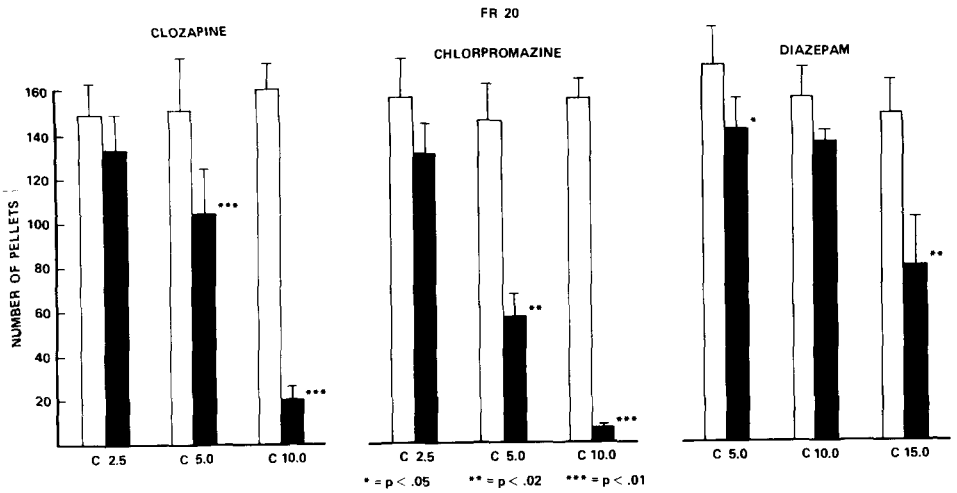

FIG. 1. Mean number of pellets (i.e., reinforcements) obtained by animals subjected to a fixed ratio 20 (FR 20) schedule under various drug or control (C) conditions. All drug doses are given in mg/kg. Each bar represents the mean of seven animals. Probability levels in this and subsequent figures refer to comparisons made between control and drug sessions using a two-tailed dependent *t*-test. The vertical lines on the bars represent the standard error of the mean.

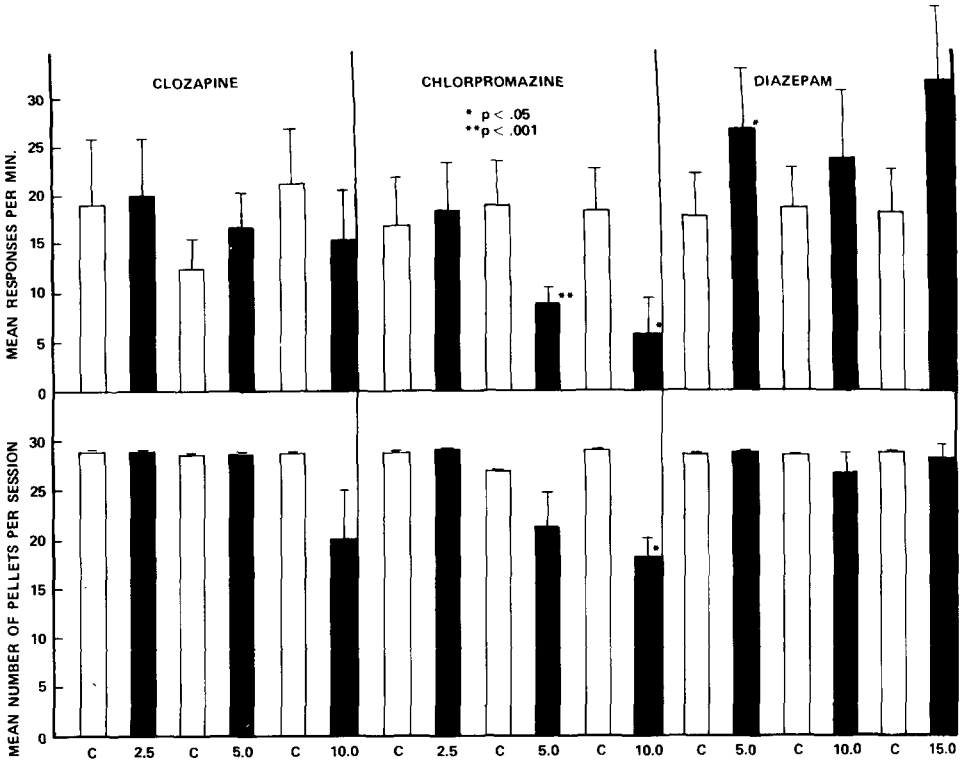

FIG. 2. Mean number of responses per minute and mean number of pellets per session recorded for a group of rats subjected to a fixed interval 2 min schedule under various control (C) and drug conditions. All drug doses are given in mg/kg.

index values are suggestive of decreased responding at the end of the interval as was seen with chlorpromazine (5.0 and 10.0 mg/kg) and clozapine (10.0 mg/kg) whereas moderately reduced indexes favor a response elevation in the middle of the interval as seen with clozapine (5.0 mg/kg) and diazepam (10.0 and 15.0 mg/kg). Under the FI schedule of reinforcement it should be remembered that in a one hr session only 29 reinforcements can be obtained per session. Thus, increments in number of reinforcements are impossible even with drugs, such as diazepam, that enhance

TABLE 1

INDEX OF CURVATURE

CLOZAPINE			CHLORPROMAZINE			DIAZEPAM		
Dose	Control	Drug	Dose	Control	Drug	Dose	Control	Drug
2.5	0.57 ± 0.03	0.57 ± 0.02	2.5	0.57 ± 0.02	0.57 ± 0.01	5.0	0.58 ± 0.05	0.50 ± 0.04‡
5.0	0.56 ± 0.04	0.47 ± 0.06*	5.0	0.56 ± 0.04	0.41 ± 0.04†	10.0	0.57 ± 0.05	0.43 ± 0.05‡
10.0	0.55 ± 0.04	0.35 ± 0.08	10.0	0.57 ± 0.04	0.24 ± 0.11‡	15.0	0.61 ± 0.02	0.45 ± 0.05*

Index of curvature of the FI 2 min curves for the three drug treatments: clozapine, chlorpromazine and diazepam. Each index represents the mean and standard error of either the control or drug treatment. Probability levels refer to comparisons made between control and drug sessions using a two-tailed dependent t-test (*$p<0.05$, †$p<0.02$, ‡$p<0.01$).

response output. Chlorpromazine at the high dose was the only drug treatment that produced a significant decrement in the number of reinforcements. Clozapine did show a drop at the high dose but this was primarily due to the two rats which exhibited marked depression.

Figure 3 presents the mean response distributions and mean amount of water consumed by animals subjected to various doses of chlorpromazine under the FI 2 min schedule. As these data indicate, chlorpromazine reduced the high response rates normally generated at the end of the 2 min period immediately preceding the presentation of a food pellet. Thus, the FI scallop was reduced in a dose-dependent manner by this compound. In addition, the amount of water consumed by the animals during the FI 2 min sessions was reliably reduced by chlorpromazine.

Figure 4 presents the mean amount of water consumed and response distributions for those animals subjected to various doses of diazepam under the FI 2 min schedule. Diazepam produced no effects on adjunctive behavior (i.e., drinking), but it did elevate response rate during the 2 min interval under all doses tested. Thus diazepam unlike chlorpromazine elevated low, moderate and high response rates that were generated during the various segments of the 2 min interval.

Finally, Fig. 5 summarizes the response distribution and drinking data for animals subjected to various doses of clozapine. Clozapine at 5.0 mg/kg elevated response rate especially during the middle phase of the 2 min interval. Under 10.0 mg/kg, however, this compound reduced response rate during the final segments of the interval immediately before the presentation of the food pellet. The last point on Fig. 5 was significant at the $p<0.05$ level. Unlike chlorpromazine, however, clozapine produced no reliable effects on drinking behavior. It could be suggested that clozapine produced changes that resembled diazepam at the lower doses and chlorpromazine at the higher doses.

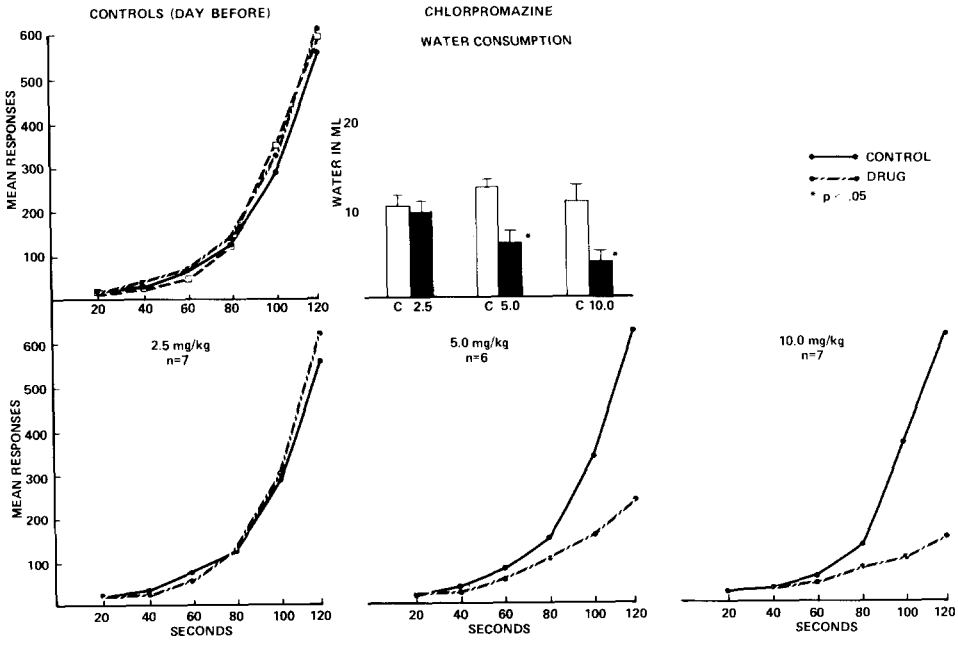

FIG. 3. Mean response distribution under the fixed interval 2 min schedule for animals subjected to various doses of chlorpromazine. The upper left hand panel of this figure plots the three control sessions immediately preceding drug administration. The bottom three panels plot drug versus control response distributions. The upper center panel summarizes mean amount of water in ml consumed by animals during the FI 2 min sessions under control or various drug dose conditions.

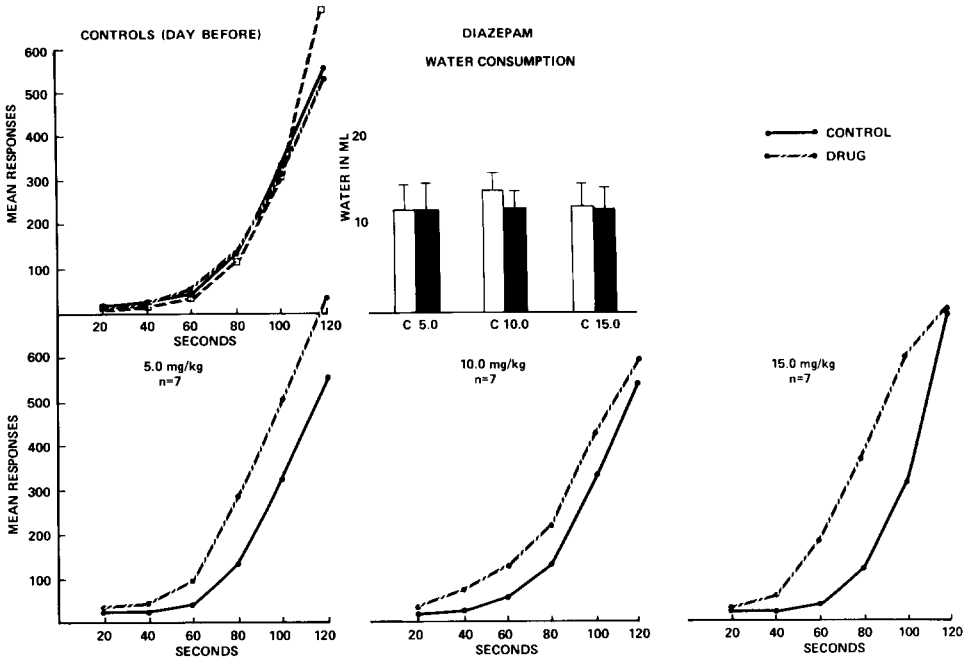

FIG. 4. Mean response distribution under the fixed interval 2 min schedule for animals subjected to various doses of diazepam. The upper left hand panel of this figure plots the three control sessions immediately preceding drug administration. The bottom three panels plot drug versus control response distributions. The upper center panel summarizes mean amount of water in ml consumed by animals during the FI 2 min sessions under control or various drug dose conditions.

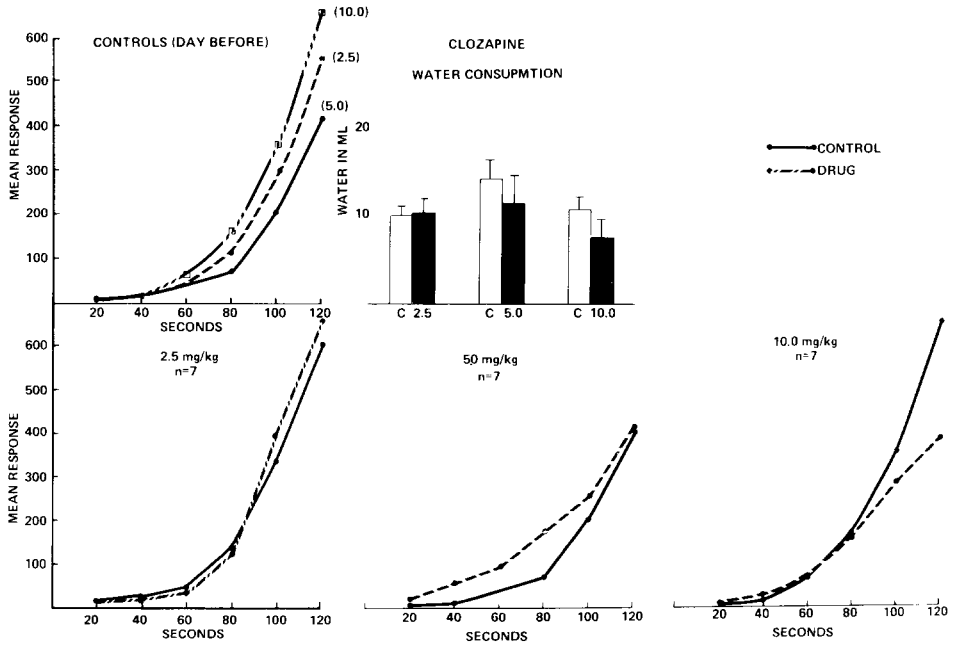

FIG. 5. Mean response distribution under the fixed interval 2 min schedule for animals subjected to various doses of clozapine. The upper left hand panel of this figure plots the three control sessions immediately preceding drug administration. The bottom three panels plot drug versus control response distributions. The upper center panel summarizes mean amount of water in ml consumed by animals during the FI 2 min sessions under control or various drug dose conditions.

Since the changes are small caution should be taken in this assumption.

DISCUSSION

The present data indicate that clozapine, chlorpromazine and diazepam produce dose-related decrements in FR 20 response rates. The two antipsychotic drugs, clozapine and chlorpromazine, appeared to be more potent in this regard than diazepam. The three compounds, however, produce dissimilar effects on response rates under the control of a FI 2 min schedule of reinforcement. Chlorpromazine reduced mean response rate in a dose-related fashion primarily by producing decrements in the high rates normally generated in the latter portion of the 2 min interval. Diazepam, on the other hand, increased rates more consistently in the middle of the interval. Clozapine at the doses tested produced effects that showed trends, although small, toward both of the above drugs. All three drug treatments did disrupt the FI scallop in different degrees as indicated by the index of curvature values. Finally, of the three drugs tested, only chlorpromazine significantly reduced the occurrence of adjunctive drinking behavior.

The present data, with regard to the decremental effects of chlorpromazine upon FR response rates, are in general agreement with previous reports. Clark [4] has noted that chlorpromazine reduced FR 25 response rates in rats as a monotonic function of doses from 0.50 to 1.5 mg/kg, I.M. Similar results were noted in regard to squirrel monkeys performing under a FR 50 schedule [6], and pigeons subjected to a FR 30 schedule [3] of reinforcement. It would thus appear that chlorpromazine produced fairly consistent decremental effects upon FR behaviors across a wide variety of species.

The data with regard to anxiolytic agents, however, are not as consistent with previous reports. Cook [7] has reported that chlordiazepoxide (10–40 mg/kg, P.O.) reduced FR 30 response rates in squirrel monkeys. Wedeking [17], on the other hand, while investigating the effects of a large number of anxiolytics on FR behaviors in rats, has noted that chlordiazepoxide (2.5, 5.0 mg/kg, IP) reliably increased in FR 25 response rates, while diazepam produced no effects at 1.0 mg/kg, IP and significant decrements at 3.0 mg/kg, IP. Several factors could account for these findings such as effects on food intake [16,18], different dose ranges in conjunction with routes of administration and species of the experimental animals. Although we can only speculate at this point, it is possible that the reductions in FR rate may be a function of the degree of sedation or ataxia produced by diazepam. If this suggestion is correct, one would expect that these side effects would produce greater response decrements under schedules that generate high baseline rates (i.e., FR) than those that produce a more moderate response output (i.e., FI).

The present data with regard to the effects of chlorpromazine and diazepam upon FI response rates are also in general agreement with the previous literature. Cook and his associates [5, 6, 7] have noted that chlorpromazine (0.3–1.2 mg/kg, P.O.) decreased responding while chlordiazepoxide (2.5–40.0 mg/kg, P.O.) enhanced the response rates of squirrel monkeys performing under a FI 10 min schedule of reinforcement. These results are confirmed by the mean response data presented in Fig. 2 of the present report. It would thus appear that diazepam produced a rate dependent effect elevating low rates under the FI schedule while decreasing higher rates generated by the FR procedure. The major discrepancy between previous reports and the present FI data concerns the effects of chlorpromazine upon the response distributions outlined in Fig. 3. In an earlier paper Clark [4] has noted that chlorpromazine (0.5–1.0 mg/kg, I.M.) not only reduces mean response rate, but also alters the response distribution during a 4 min fixed interval schedule in rats. Clark also noted that chlorpromazine increased the relatively low rates during the initial portion of the fixed interval, while reducing the higher rates generated at the end of this interval, especially under the higher doses of the drug. Similar drug results on response distributions have been noted in pigeons subjected to FI schedules of reinforcement [3,13]. These results are clearly unlike those seen in Fig. 3 where chlorpromazine never elevated response rates in the initial segments of the fixed interval. It is possible that since other routes of drug administration and fixed interval schedules were utilized in the previous reports direct comparisons should not be made between earlier results and the present data. Response rates during the initial portion of a four minute fixed interval are somewhat lower than during a two minute interval. Thus, elevations in response rate after chlorpromazine administration may be more easily detected in FI 4 min rather than the FI 2 min schedule utilized in this report.

Even with the above discrepancies in mind, however, it appears clear that chlorpromazine and diazepam produce opposing behavioral effects in animals subjected to FI schedules of reinforcement. The antipsychotic, chlorpromazine, produced reliable decrements in response rate particularly during the latter portions of the fixed interval, while the anxiolytic, diazepam, produced consistent elevations in rate throughout the interval. Clozapine, on the other hand, elevated rates when given in a moderate dose, while decreasing rates especially during the later portions of the fixed interval when administered at the highest dose. The differential dose-related effects noted in response to clozapine suggest that FI schedules of reinforcement may be useful in outlining the behavioral profile of an agent that possesses antipsychotic properties with little extrapyramidal liability.

The use of a measure of adjunctive behavior to assess drug effects in the present paper was suggested by an earlier report by Falk [11] which noted that both metamphetamine (0.5 mg) and pentobarbital (2.0 mg) decreased adjunctive drinking without noticeably affecting lever pressing under a variable interval schedule. These data indicated that changes in adjunctive behavior might be a more sensitive index of drug activity than schedule controlled behaviors. Unfortunately, the present data do not support this optimistic assumption. Clozapine and diazepam had no significant ($p<0.05$) effect on adjunctive (i.e., drinking) behavior even though clozapine did exhibit a measurable drop in water intake. Only chlorpromazine was able to produce effects on both adjunctive and schedule-controlled behaviors at comparable doses. Earlier data from other laboratories also suggests that adjunctive behavior may not be useful as a sensitive index of drug activity. McKearney [14] has reported that both chlordiazepoxide and methamphetamine had little effect on overall adjunctive licking except for decreases at the highest dose tested. Likewise, Smith and Clark [15] have noted that chlorpromazine and chlordiazepoxide were both able to

reliably affect lever-pressing behavior on a DRL schedule (i.e., differential-reinforcement-of-low-rate) while two types of adjunctive behaviors (i.e., licking and running) were differentially affected by the drugs in different animals. Bacotti and Barrett [2] were able to show an increase in water consumption in three out of four rats in a multiple FR-FI schedule with chlordiazepoxide. Thus, it appears that no consistent pattern of drug effects on this behavior has yet been established across a group of animals. The above data suggest that adjunctive drinking behavior may not be useful at this time in assessing the behavioral profiles of various types of compounds.

To summarize, the present data indicate that FR schedules of reinforcement might be useful in assessing the side-effect liability (i.e., sedation, ataxia) of various drugs. Fixed interval schedules, on the other hand, might allow one to differentiate anxiolytic from antipsychotic activity and possibly to obtain a differential behavioral profile of clozapine. More compounds (i.e., antipsychotic, anxiolytic, etc) will have to be tested before the value of these procedures in delineating the specific properties of drugs can be completely ascertained.

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