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Effects of Imipramine, Bupropion, Chlorpromazine, and Clozapine on Differential-Reinforcement-of-Low-Rate (DRL) > 72-Sec and > 36-Sec Schedules in Rat

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ABSTRACT

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The hypothesis that a DRL schedule requiring a long pause (> 72 sec) specifically identifies antidepressant drugs was evaluated in rats pressing a lever for food reinforcement. The tricyclic antidepressant imipramine decreased responses and increased reinforcements as previously shown. However, the antipsychotics chlorpromazine and clozapine had a similar effect, as did prefeeding, and the atypical antidepressant bupropion increased responses and decreased reinforcements. Because mean baseline reinforcement rate was lower than in previous studies showing drug class specificity, the DRL requirement was reduced to > 36 sec and the drugs were tested again; effects were qualitatively similar to those in DRL > 72 in the first part of the study. The results suggest that a drug-induced reduction in responses could account for the increase in reinforcements, that if drug class specificity exists it may occur only when baseline response and reinforcement rates are confined to a narrow range, that the failure of previous studies to show antidepressant type effects with nonantidepressants could have resulted from choice of drugs and doses, and that the type of reinforcer could be an important factor.

Key words: DRL schedules, antidepressants, imipramine, chlorpromazine, clozapine, bupropion, diphenhydramine, rat

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INTRODUCTION

Under a differential-reinforcement-of-low-rate (DRL) schedule, a response is reinforced only if a specified period has elapsed since the previous response [Zeiler, 1977]. There is no rule governing the minimum interresponse time (IRT) in a nominally DRL schedule; the expected effects of such a schedule, low response rates, may in fact be high response rates if the pause required between responses is very short. The term DRL has been criticized also because it implies something more than "the simple description of the prescription for reinforcer delivery," and a terminology based upon the IRT requirement has been suggested [Zeiler, 1977]. However, the term DRL has been widely used in the literature and is retained here for convenience.

A DRL schedule requiring a relatively long pause, > 72 sec, was proposed as a screening method for antidepressant drugs. Seiden and co-workers trained male albino rats to press a lever for water on a DRL > 72-sec schedule and found that intraperitoneal (i.p.) injection of most clinical antidepressants tested decreased responses and increased reinforcements in a dose-dependent manner. The tricyclic antidepressants imipramine, desipramine, chlorimipramine, protriptyline, nortriptyline, amitriptyline, and doxepin [McGuire and Seiden, 1980; O'Donnell and Seiden, 1983]; the monoamine oxidase inhibitors (MAOIs) isocarboxazid, iproniazid, phenelzine, and tranylcypromine [O'Donnell and Seiden, 1982, 1983]; and the atypical antidepressants iprindole, mianserin [O'Donnell and Seiden, 1983], trazadone, and fluoxetine [Seiden, 1983] decreased responses and increased reinforcements. However, the atypical antidepressant nomifensine, which has been shown to increase locomotor activity in rodents [Gerhards et al., 1974], increased responses and decreased reinforcements [O'Donnell and Seiden, 1983]; and the atypical antidepressant bupropion, which also has been shown to increase locomotor activity in rodents [Soroko et al, 1977] and to generalize to stimulant drugs in a drug discrimination paradigm [Jones et al., 1980], had similar effects except at one low dose, where it resembled most other antidepressants [Seiden, 1983]. Several nonantidepressants did not significantly increase reinforcements: alcohol, chlordiazepoxide, morphine, pentobarbital [O'Donnell and Seiden, 1982], chlorpromazine, and diphenhydramine [O'Donnell and Seiden, 1983].

A more parsimonious explanation of the effect of nonstimulant antidepressants in DRL would be that it results not from a specific "antidepressant" drug action but from a nonspecific reduction in response rate and concomitant increase in reinforcement rate—that is, an essentially behavioral rather than pharmacological effect. The paucity of convincing data on response-decreasing nonantidepressant drugs in the DRL > 72 literature suggests that this alternative hypothesis warrants further consideration. Neither chlordiazepoxide nor pentobarbital at the doses tested decreased response rate significantly; in fact, they increased it [O'Donnell and Seiden, 1982]; therefore the data do not apply directly to the alternative hypothesis. One dose of morphine, 20 mg/kg, decreased responses nonsignificantly in terms of mean percent of control ± SE (54.9 ± 26.7) and increased reinforcements nonsignificantly (127.9 ± 59.5) [O'Donnell and Seiden, 1982], an effect qualitatively similar to that of tricyclic antidepressants. For two reasons, the data on diphenhydramine do not constitute a good test of whether the DRL model can reject nonantidepressant drugs: First, the effect was variable, a significant decrease in responses occurring at 10 mg/kg, a nonsignificant increase at 20 mg/kg, and a nonsignificant decrease at 40 mg/kg, with concomitant decreases in reinforcements (at 20 and 40 mg/kg) that did not reach significance; second, the LD_{50} i.p. has been determined as 82 mg/kg [Barnes and Eltherington, 1973], and the ED_{50} for seizure is probably between 40 and 60 mg/kg (see "Results"; O'Donnell and Seiden [1983] observed tremor at 40 mg/kg), so that a reduction in responses could be secondary to toxicity—to a preconvulsive state that would interfere with emission of the operant—rather than to the sort of reduction produced by other response-decreasing drugs. The three doses of chlorpromazine tested—1.0, 2.0, and 4.0 mg/kg—may be inadequate to demonstrate rejection of nonantidepressant drugs that reduce general activity: The low dose had no effect, the high dose reduced responses by nearly 60%

to a level at which no antidepressant in any of the studies cited showed a significant increase in reinforcements, and the middle dose reduced responses significantly and increased reinforcements nonsignificantly [O'Donnell and Seiden, 1983]. Of the response-decreasing nonantidepressants tested, only alcohol convincingly reduced responses without increasing reinforcements [O'Donnell and Seiden, 1983].

The purpose of the present study was to assess the specificity of the DRL > 72 as a screening method for antidepressant drugs. The research hypotheses were 1) that in at least some cases the reduction of response rate is sufficient to increase reinforcement rate independently of any known antidepressant properties of the agents used and 2) that at least one clinically effective antidepressant, in addition to nomifensine, produces an effect opposite to that of the response-decreasing antidepressants previously tested. Imipramine was tested as a reference compound. Chlorpromazine, clozapine, and prefeeding were administered to test hypothesis 1, bupropion to test hypothesis 2. Both hypotheses were confirmed in DRL > 72.

Because mean response rate was somewhat higher and mean reinforcement rate lower than rates found in the studies that proposed the high-value DRL schedule as a screening method for antidepressants, the DRL requirement was reduced to > 36 sec and the drugs (except diphenhydramine) were retested. Both hypotheses were again confirmed.

METHODS

Subjects

Ovariectomized female Long-Evans rats from Blue Spruce Farms, Altamont, New York, served as subjects. They were approximately 5 months old at the beginning of training. Females were chosen because they are smaller (therefore requiring less compound on a mg/kg basis) and less aggressive (therefore easier to house and handle) than males. They were ovariectomized to prevent the fluctuation of sex hormone levels, which might affect behavior. Two subjects died after a high dose of diphenhydramine in the DRL > 72 portion of the study and were replaced by two standby subjects for the DRL > 36 portion.

Apparatus

Training and testing were done in two identical Coulbourn operant chambers inside light- and sound-attenuating Coulbourn enclosures. One wall of the chamber held a standard lever manipulandum at the lower left, a feeder bin at the lower center, and a house light at the upper center. A standard Coulbourn food dispenser delivered one 45-mg BioServ pellet for each correct response. Control and data acquisition were done by a Data General NOVA 3 minicomputer via an INTERACT interface.

Procedure

Naive subjects were deprived of food 24 h before the first of several 15-h nightly sessions of initial training, in which a single DRL value was in effect throughout a session. With minor exceptions, a DRL > 5 was in effect for the first two sessions, and advancement to DRL > 10, > 20, > 40, and > 72 in subsequent sessions was contingent upon the subject's receiving at least 100 reinforcements in a session. The median number of 15-h nightly training sessions required to reach DRL > 72 was 9. Subjects were then put on a regimen of daily 1-h DRL > 72 sessions 6 days per week, with 1 h of access to food (Wayne Lab Blox) postsession and 1 h on Sundays. During the first 2 months of training, supplemental food was given to some subjects to maintain body weight; during the third month of training, the 4 months of drug testing on DRL > 72, and the subsequent 2 months on DRL > 36, mean body weight remained relatively stable on the 1-h-per-day feeding regimen, although some individual fluctuations occurred. Subjects were maintained on a reverse light-dark cycle (lights on1600–0400) and were run during the dark period. Drug injections were given 1 h presession on Tuesdays and Fridays.

Drugs

Imipramine HCl (Tofranil) was a gift from CIBA Pharmaceutical Company, chlorpromazine HCl (Thorazine) from SmithKline Corporation, clozapine from Sandoz Pharmaceuticals, diphenhydramine (Benadryl) from Parke, Davis. Bupropion (Wellbutrin) came from Burroughs Wellcome Co. All compounds except clozapine were dissolved in isotonic saline; clozapine was suspended in 0.5% methyl cellulose. Injections were given i.p. in a volume of 1 ml/kg body weight. Each subject received each dose once and saline once. All doses of a drug were tested in a block of four sessions: Six subjects received one dose and four subjects another dose on Tuesday of a week, and this order was reversed on Friday; the remaining two doses were given similarly the next week to complete the dose-effect curve. The treatments were tested in the following order: imipramine, bupropion, clozapine, saline, prefeeding, chlorpromazine, diphenhydramine in DRL > 72; chlorpromazine, bupropion, imipramine, saline, clozapine in DRL > 36.

Data

Total number of responses and total number of reinforcements per session were counted. For the DRL > 72 portion of the study, IRTs were gathered into 7.2-sec bins (one-tenth the IRT requirement for reinforcement) for the first 108 sec, with a single bin for IRTs greater than 108 sec. For the DRL > 36 portion, IRTs were gathered into 3.6-sec bins (one-tenth the IRT requirement for reinforcement) for the first 54 sec, with a single bin for IRTs greater than 54 sec. Mean number of responses and mean number of reinforcements per session for each subject for the two nontreatment sessions preceding and the two nontreatment sessions following a treatment day were used as control values for that treatment, with minor exceptions. The differences between treatment and control values were tabulated and subjected to the t-test for dependent samples; criterion for significance was $P < .05$. If a subject emitted few or no responses after an injection, its data for that session were not used in calculating group mean effects; this happened in three cases during the DRL > 72 portion, one subject each after imipramine 20 mg/kg, clozapine 20 mg/kg, and chlorpromazine 2.0 mg/kg. The IRT distributions were converted to percentages by dividing responses in each bin by total responses and plotting the results as histograms for visual comparison of drug sessions to vehicle session. Over the course of testing in the DRL > 36 portion of the study, one subject showed a radical increase in baseline reinforcements per session (range 12.3 to 59.0), to a rate that imipramine failed to increase; data for this subject were cast out of calculations. Another subject was injured part of the way through the DRL > 36 portion; data up to that point were considered valid.

RESULTS

DRL > 72

The left half of Table 1 gives mean baseline control responses and reinforcements per session for each subject over the entire DRL > 72 portion of the study. Figure 1 shows the effects of saline and drug injections and of prefeeding on responses and reinforcements. (Note that in Fig. 1 data for DRL > 72 are indicated by circles, for DRL > 36 by triangles.) Saline had no significant effect. The tricyclic antidepressant imipramine decreased responses and increased reinforcements in a dose-dependent manner. However, the nonantidepressants chlorpromazine and clozapine had the same effect. Allowing subjects free access to food for 17 h presession produced an effect that was qualitatively and quantitatively similar to the effects of the highest doses of the tricyclic antidepressant and the two antipsychotics, a decrease in responses and an increase in reinforcements. The atypical antidepressant bupropion had an effect opposite to that of the tricyclic antidepressant: It increased responses and decreased reinforcements. The antihistamine diphenhydramine significantly increased reinforcements at

TABLE 1. Mean Baseline Control Responses and Reinforcements Per Session

	DRL > 72		DRL > 36	
Rat No.	Responses	Reinforcements	Responses	Reinforcements
55	86	7.5	134	25.1
56	157	6.8	205	32.1
57[a]	123	5.0	—	—
58[a]	154	3.2	—	—
59	232	2.6	383	8.7
60	158	5.6	208[b]	36.5[b]
66	124	4.7	223	16.0
67	148	4.5	242	16.6
68	134	3.8	179	17.3
69	126	7.7	206	21.2
70[a]	—	—	394	24.2
83[a]	—	—	273	8.4
Mean	144	5.1	249	18.8
SE	12	0.5	29	2.6

[a]Nos. 57 and 58 died at the end of the DRL > 72 portion of the study and were replaced by Nos. 70 and 83 for the DRL > 36 portion.
[b]On DRL > 36, baseline reinforcement rate changed radically for No. 60 (range 12.3–59.0); data for this subject in DRL > 36 were cast out.

40 mg/kg; when given to four subjects at 60 mg/kg it induced seizure in three of them, two of which died.

The IRT distributions for three subjects are shown in Figures 2–5. Subjects were selected on the basis of baseline reinforcement rate: No. 55 was high, No. 67 moderate, No. 59 low (individual values are given in Table 1). Some intrasubject variability in drug effect is evident, but in general the response-decreasing drugs imipramine, chlorpromazine, and clozapine tended to shift the frequency distribution to the right as a function of dose—decreasing the percentage of short IRTs and increasing the percentage of long ones—whereas the response-increasing drug bupropion had the opposite effect. The shape of the control IRT distribution seemed to have little qualitative or quantitative bearing upon drug effects: The antipsychotics shifted the distribution to the right and increased the percentage of reinforced responses in the subject with a normal distribution (No. 55, 7.5 reinforcements per session in baseline) as much as or more than in the subject with a baseline distribution skewed sharply to the left (No. 59, 2.6 reinforcements per session in baseline). The tricyclic antidepressant could not be distinguished from the antipsychotics on the basis of effects upon IRT distribution.

DRL > 36

In the DRL > 72 studies mentioned in the introduction, a typical set of baseline mean values for reinforcements and responses per session was 15.9 ± 2.9 and 85.1 ± 7.9 [O'Donnell and Seiden, 1982] (mean control values for reinforcements per session in this reference ranged from 12.2 ± 3.0 to 18.5 ± 2.9). Under DRL > 72 in the present study, comparable baseline values were 5.1 ± 0.5 and 144 ± 12 (Table 1). The effects of several nonantidepressants had been shown to depend in part upon the baseline generated by the DRL > 72 schedule; for example, "chlorpromazine did increase the reinforcement rate of some rats with low to moderate control reinforcement rates" [O'Donnell and Seiden, 1983]. Therefore, it seemed possible that the effects of chlorpromazine and clozapine in DRL > 72 could have resulted from the comparatively low baseline reinforcement rate and would disappear if the baseline rate were raised.

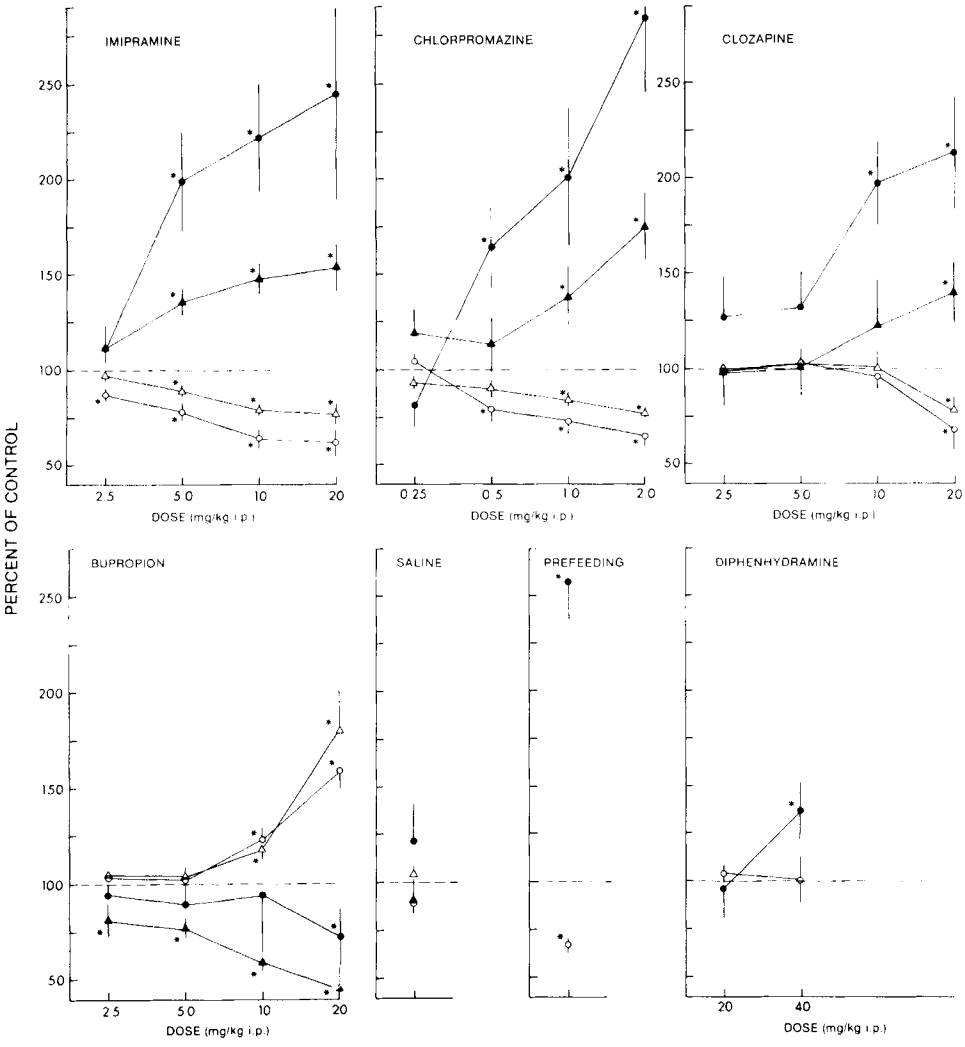

Fig. 1. Effects of five drugs, vehicle, and prefeeding on responses (open symbols) and reinforcements (filled symbols) per session. Circles denote differential reinforcement of low rate (DRL) > 72, triangles DRL > 36. * Denotes significant difference from baseline control, $P < .05$.

When the DRL value was reduced to >36 sec, mean baseline reinforcement rate immediately increased, and it remained quite stable throughout the initial 11 days of baseline training and the period of drug testing (with the exception of the one subject mentioned above). The right half of Table 1 gives mean baseline control responses and reinforcements per session for each subject. The group mean for baseline reinforcements, 18.8 ± 2.6, was at the upper limit of the group means for DRL > 72 found in a previous study, 18.5 ± 2.9 [O'Donnell and Seiden, 1983]. However, group mean responses per session, 249 ± 29, was much higher than values found in the literature and in the DRL > 72 portion of the present study, presumably because in these subjects the sudden increase in reinforcement rate produced a dynamic increase in response rate.

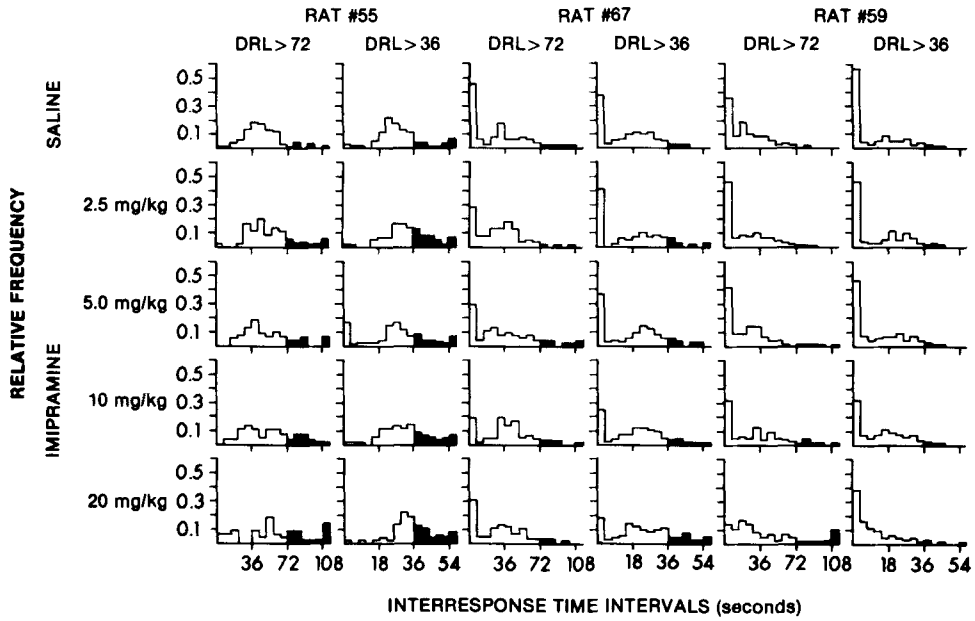

Fig. 2. Interresponse time (IRT) distributions for DRL > 72 and DRL > 36 after vehicle or imipramine. The height of a bar represents percentage of responses occurring in that bin (7.2 sec for DRL > 72, 3.6 sec for DRL > 36). The last bin in each graph contains all IRTs greater than 1½ times the DRL value. Filled area represents reinforced responses.

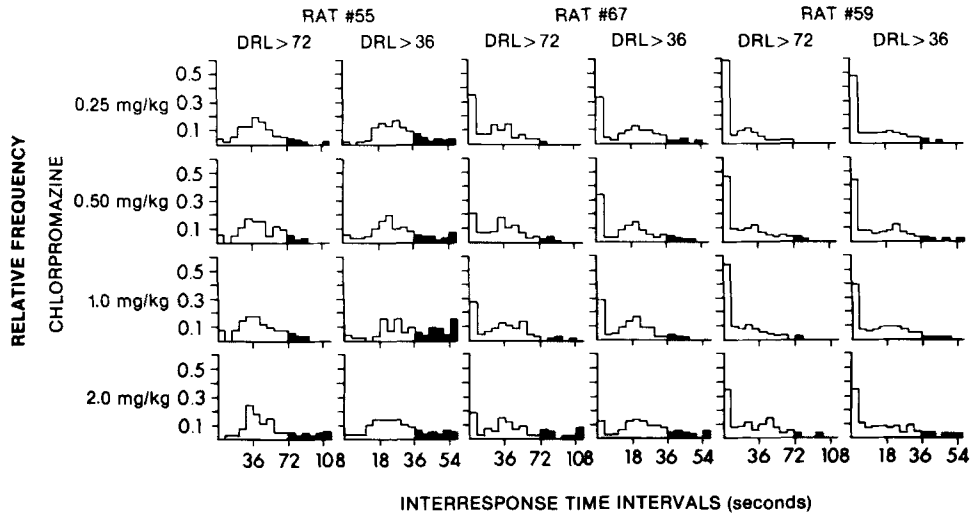

Fig. 3. IRT distributions for chlorpromazine. All other information as in Figure 2.

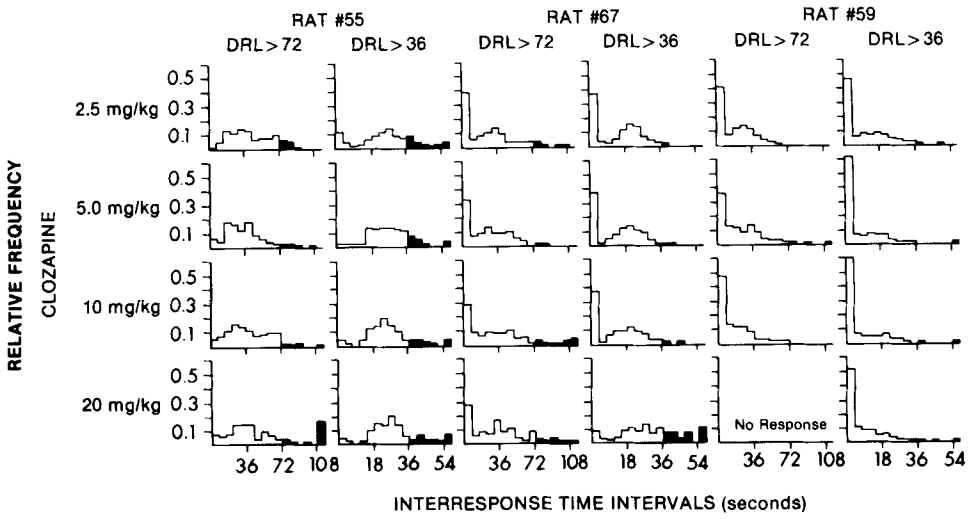

Fig. 4. IRT distributions for clozapine. All other information as in Figure 2.

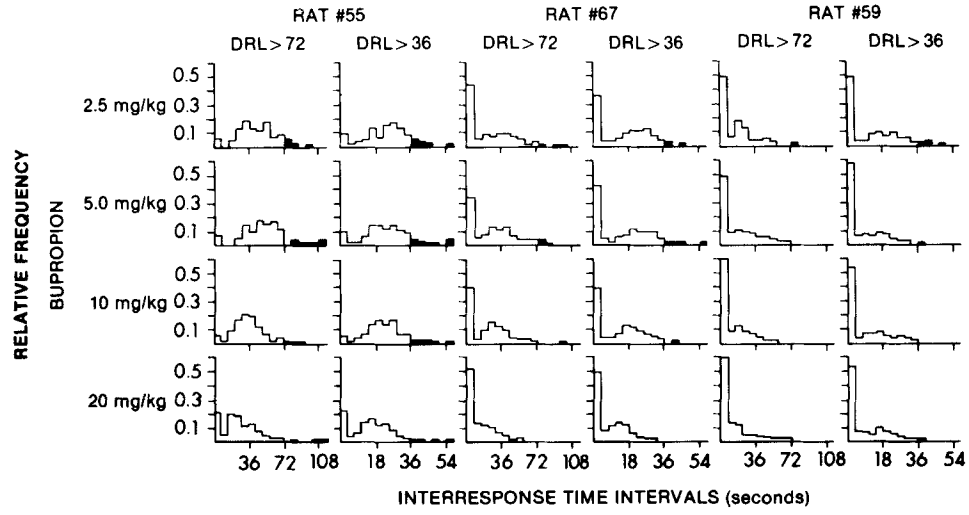

Fig. 5. IRT distributions for bupropion. All other information as in Figure 2.

Figure 1 (triangles) shows the effects of drugs on responses and reinforcements in DRL > 36. In general, behavior was changed somewhat less by the three response-decreasing drugs and somewhat more by the stimulant drug than was behavior under DRL > 72, but qualitatively the effects on responses and reinforcements under the two schedules were similar. The effects on IRT distributions, shown in Figures 2–5, also were qualitatively similar.

DISCUSSION

The tricyclic antidepressant imipramine decreased responses and increased reinforcements in DRL > 72 and DRL > 36, confirming previous results for tricyclic antidepressants, MAOIs, and nonstimulant atypical antidepressants in DRL > 72. Two nonantidepressant drugs, the phenothiazine antipsychotic chlorpromazine and the atypical antipsychotic clozapine, also decreased responses and increased reinforcements in DRL > 72 and DRL > 36, contradicting previous results or the interpretation of those results for response-decreasing drugs. Prefeeding decreased responses and increased reinforcements in DRL > 72, mimicking previous results for nonstimulant antidepressants. The atypical antidepressant bupropion, which has some stimulant properties in rodents, increased responses and decreased reinforcements in DRL > 72 and DRL > 36, confirming previous results for the stimulant type atypical antidepressant nomifensine and for high doses of bupropion but contradicting previous results for one behaviorally marginal dose of bupropion, 5.0 mg/kg, as reported in an abstract. The drug effects that disagreed with previous results were, on the whole, large, dose-related, and statistically convincing. Several factors could account for these differences.

The methods of the present study differed from the methods of previous studies in several ways: use of ovariectomized female Long-Evans rats instead of male Sprague-Dawleys, food instead of water reinforcement, a more gradual increase in the DRL value during initial training, a 6- instead of a 7-day week, a 12-h light/12-h dark cycle with testing during the dark portion instead of a 16-h light/8-h dark cycle with testing during the light portion. Most of these factors seem unlikely to be determinants of profound differences in drug effects: Sex, strain, and light/dark cycle might have small quantitative influences but would be low on a list of priorities as critical variables to be controlled. Initial training procedure differed in apparently minor ways, and baseline behavior did not differ qualitatively. Type of reinforcer could be more important because of pharmacological action (for example, dry mouth as a result of the anticholinergic effect of tricyclic antidepressants) or consummatory topography; however, an explanation of pharmaco-behavioral side-effects would be quite complex (for example, not all response-decreasing antidepressants have been shown to possess strong anticholinergic effects), and the topography of drinking during a 4-sec dipper presentation would have to be differentiated in some critical way from that of eating a 45-mg pellet from a bin, both of which the subject has 72 (or 36) sec to accomplish before the next reinforced response.

Baseline reinforcement rate could be a factor, especially since in previous studies several nonantidepressants decreased responses and increased reinforcements in individual subjects with low baseline reinforcement rates—alcohol, morphine, pentobarbital [O'Donnell and Seiden, 1982], and chlorpromazine [O'Donnell and Seiden, 1983]. However, chlorpromazine and clozapine still decreased responses and increased reinforcements for group data in the present study when a reduction of the DRL value to > 36 sec had increased mean reinforcement rate to the level found in previous studies. Whether the history of the subjects and the relatively high response rate in DRL > 36 could account for this effect is a question to be explored.

The authors of previous work claimed that nonstimulant antidepressants could be distinguished from chlorpromazine by the fact that the antidepressants shifted the IRT distribution to the right, whereas chlorpromazine only disrupted the pattern, although this difference is not readily apparent from the data offered [Fig. 11 in O'Donnell and Seiden, 1983]. In the present study the IRT changes produced by imipramine and chlorpromazine were qualitatively indistinguishable. The authors also claimed that "the performance-improving and disruptive effects of chlorpromazine are less separated in dose than are the effects for the antidepressants" [O'Donnell and Seiden, 1983]. However, their dose range for tricyclic antidepressants was 2.5 to 20 mg/kg, an eightfold difference, whereas their dose range for chlorpromazine was 1.0 to 4.0 mg/kg, a fourfold difference. The claim that IRTs and breadth of dose range can be used to achieve drug class specificity needs further support.

One explanation for the absence of a clear increase in reinforcements by chlorpromazine in previous work and the presence of clear increases in reinforcement by chlorpromazine and clozapine in the present study is that the dose range in previous testing was inadequately explored. As stated in the introduction, chlorpromazine was previously tested at three doses: The lowest produced no effect, the highest drove response rate down to a level at which no antidepressant was able to increase reinforcements significantly, and the middle dose produced changes in the same directions as did nonstimulant antidepressants. Furthermore, the results from all other nonantidepressants tested, except alcohol, are open to the objections raised in the introduction: In no case was there a clear, significant, dose-related decrease in response rate in conjunction with a failure to increase reinforcements.

In summary, treatments that decreased responses increased reinforcements, and treatments that increased responses decreased reinforcements, irrespective of drug class. Whether the disagreement with previous data resulted from differences in strain, sex, type of reinforcement, baseline response and reinforcement rate, or some procedural difference remains to be determined. The most parsimonious explanation of the effects seen here and in previous studies would be behavioral rather than pharmacological: In the absence of gross effects such as the tremor produced by diphenhydramine or the ataxia produced by alcohol, a simple reduction in response rate could account for an increase in reinforcement rate, and a simple increase in response rate could account for a reduction in reinforcement rate.

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