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# A parametric analysis of punishment frequency as a determinant of the response to chlordiazepoxide in the Vogel conflict test in rats

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**Abstract**

The Vogel conflict test has been widely used as a methodology for detecting anxiolytic-like effects of drugs with a broad spectrum of pharmacological activities. Despite widespread acceptance of the Vogel assay as a preclinical predictor of efficacy for anxiolytic-like compounds, detailed parametrics have not been reported on the optimization of this assay to determine how the schedule of reinforcement, the rate of responding and the frequency and temporal distribution of punishing events determine drug effect. The current report documents results of a systematic study of the relationship between number of shocks delivered and efficacy of the prototypical 1,4-benzodiazepine anxiolytic chlordiazepoxide (CDAP) in rats. Under this procedure, water-deprived rats were given access to water and during the later part of this access period, contacts with the drinking tube produced a brief electric shock. CDAP (5–20 mg/kg, i.p.) was first tested under a fixed-ratio 20 response schedule (every 20 th lick produced shock delivered via the sipper tube). CDAP produced dose-dependent increases in punished licking to approximately 275% of control at 20 mg/kg. Increasing the number of shocks during the first ten responses of the punishment component decreased the number of licks made under vehicle control conditions. The frequency of shock delivery produced both quantitative and qualitative changes in the effects of chlordiazepoxide ranging from no effect to 7000% increases in responding. The effects of chlordiazepoxide were dependent both on the control rate of responding and, independently, on the frequency of shock deliveries. Parametric variation under the Vogel conflict test may be useful in comparing the efficacy of novel approaches to the treatment of anxiety disorders.
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*Keywords:* Vogel conflict test; Chlordiazepoxide; Shock frequency; Rat

## 1. Introduction

Since the Vogel conflict test was first introduced (Vogel et al., 1971), it has become widely used for the detection of anxiolytic-like activity (e.g. Millan and Brocco, 2003). The Vogel procedure was designed to reduce training time and retain the predictive utility of the Geller–Seifter conflict test (Geller et al., 1962). However, unlike the Geller–Seifter conflict procedure where behavior is suppressed by punishment and challenged with pharmacological agents, drug effects under the Vogel procedure are evaluated under conditions in which punishment contingencies are introduced for the first time. Drug effects are thus assessed for their ability to prevent the suppressive effects of punishment, whereas under the Geller–Seifter procedure, drug effects are assessed in terms of their ability to reverse suppression of behavior decreased by punishment. Nonetheless, although direct comparisons have not been made, the two methods generally produce comparable results with compounds producing anxiolytic-like effects in both assays (e.g. Millan and Brocco, 2003; Witkin, 2002).

Under the Vogel test, water-deprived animals are given access to water. After a short period of drinking, all or some licks on the drinking spout produce mild electrical shock. In addition to variations on the frequency of shock delivered upon drinking, a host of differences exist in the procedures reported across laboratories. There are differences in the species (mice and rats), strains of animals, different methods of water deprivation, water access periods, location of shock delivery (drinking spout vs. grid floor), and schedules of punishment (e.g. Millan and Brocco, 2003). In addition, although most investigators have used an animal only once in this test, others have used the same animals over several pharmacological challenges (Vanover et al., 1999). The procedure has also shown

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Table 1
Experimental parameters studied

| Number of shocks delivered | Licks shocked |
|---|---|
| FR20 | Every 20 th |
| 1 | 1 |
| 2 | 1 and 10 |
| 3 | 1, 5 and 10 |
| 4 | 1, 3, 7 and 10 |
| 5 | 1, 3, 5, 7 and 10 |
| 6 | 1, 2, 3, 5, 7 and 10 |
| 7 | 1, 2, 3, 5, 7, 8 and 10 |
| 8 | 1, 2, 3, 4, 5, 7, 8 and 10 |
| 9 | 1, 2, 3, 4, 5, 6, 7, 8 and 10 |
| 10 | 1 to 10, inclusive |

utility when food rather than water is used to maintain responding (Witkin et al., 2004).

Despite the widespread use of the Vogel conflict procedure for detection of anxiolytic-like activity, there have been few studies that have reported results of assay optimization experiments although, as already noted, most studies utilize variations on the original conditions without parametric analysis of the effects on drug actions (e.g. Millan and Brocco, 2003). One study found modifying the level of shock intensity produced greater anxiolytic-like effects of buspirone at lower shock levels versus a higher intensity shock (Meneses and Hong, 1993). This is in accord with findings in pigeons using the Geller–Seifter procedure (Benvenga and Leander, 1996). However, there have been no systematic reports evaluating the effect of shock frequency or shock distribution on drug effects. The purpose of the current study was to evaluate the relationship between the number of shocks delivered and efficacy of the prototypical benzodiazepine, chlordiazepoxide (CDAP). First, we evaluated effects of CDAP under the FR20 protocol, where every 20 th lick during the punishment component produced shock. Second, shock was systematically varied during the first 10 licks during the punished component until all ten licks produced shock. Effects of CDAP were studied in a dose–response manner across all of these variations.

## 2. Materials and methods

### 2.1. Subjects

Groups of twenty four to thirty two naive adult male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN), weighing between 200 and 300 g were used. Each group was tested with CDAP three times and then a new group was established. Animals were removed from shipping crates and placed into Plexiglas cages (4 per cage) containing Sani-Chips bedding material (Harlan/Teklad, Madison, WI, USA), and given free access to Lab Diet #5001 for rodents (PMI Nutrition International Inc., St. Louis, MO, USA). Water was withheld for 20–24 h prior to the first training session. A 12-h light/dark cycle (lights on 6am, lights off 6pm) was maintained, and all experimental sessions were conducted during the light phase of the cycle. Experimental sessions were conducted between noon and 4pm. All work was done in accordance with "The principles of laboratory animal care" (NIH publication No. 85-23, revised 1985). Furthermore, all research protocols were approved by an internal Animal Care and Use Committee.

### 2.2. Apparatus

The experiments were conducted using twelve operant behavior test chambers (ENV-007, Med Associates Inc., Georgia, Vermont, USA, dimensions 30.5×24.1×29.2 cm). The test chambers were contained within light and sound attenuating shells in which white masking noise and a ventilating fan operated. On the front wall of the chamber, a food trough was mounted 2 cm off the grid floor on the centerline. Two response levers were centered 8 cm off the centerline and 7 cm off the grid floor. Three lights were located above each response lever at 15 cm off the grid floor. Responding on the levers was without consequences for all sessions. On the rear of the chamber, a sipping tube was mounted 3 cm off the grid floor and 3 cm from the door and extended 1.5 cm into the chamber. Standard non-ball bearing type sipping tubes (outer diameter 0.8 cm) were used. The sipping tube was wrapped with electrical tape exposing only about 0.5 cm of the tip of the tube; this insulation was used to prevent the circuit from being completed if the animals were holding/touching the sipping tube. A lick was defined as a single discreet contact with the water sipper tube. All events were controlled and licking data was recorded by a Compaq computer running MED-PC Version IV (Med Associates Inc., Georgia, Vermont, USA).

### 2.3. Sipper tube training

Rats were placed into individual operant chambers on days 1 and 2 with white noise and the houselight illuminated, and allowed to drink for a total of 6 min after the first lick was made. The 6 min were divided into two components; the first 3 min were recorded as the unpunished component, the second 3 min were recorded as the punished component. During the two training days no shock was delivered in the punished

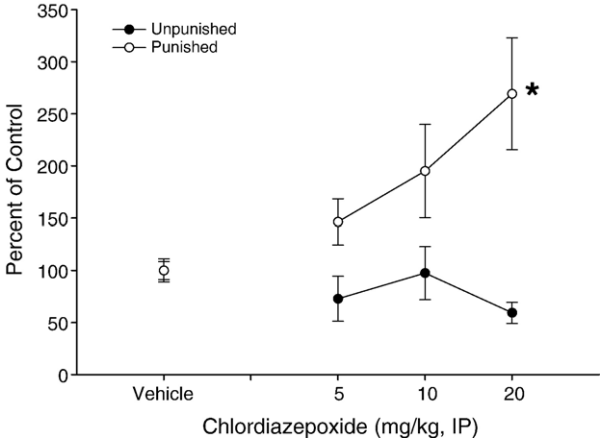


Fig. 1. Effects of chlordiazepoxide under the FR20 Vogel procedure. Each point represents the mean, and vertical lines are the S.E.M. ($n$=6–8 for each point). *Significantly different than vehicle control, $P<0.05$ using ANOVA followed by Dunnett's test.

Table 2
Number of responses and percentage of vehicle control rate in the punishment component for vehicle control and chlordiazepoxide

| Treatment | FR20 | 1 shock | 2 shocks | 3 shocks | 4 shocks | 5 shocks | 6 shocks | 7 shocks | 8 shocks | 9 shocks | 10 shocks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 91±10 | 372±54 | 241±56 | 139±47 | 6.7±1.6 | 14.5±6.3 | 7.7±0.7 | 4.8±1.0 | 6.4±1.4 | 4.0±0.5 | 6.3±1.2 |
| CDAP | | | | | | | | | | | |
| 5 | 134±20 | 286±83 | 224±54 | 340±86 | 209±72 * | 310±86 * | 279±69 * | 65±43 | 188±77 | 137±72 | 129±58 |
| 10 | 178±41 | 286±53 | 247±41 | 240±59 | 197±63 * | 265±76 | 235±63 * | 151±87 | 167±64 | 184±75 | 115±82 |
| 20 | 246±49 * | 193±40 | 353±79 | 289±86 | 117±67 | 317±78 * | 270±52 * | 334±109 * | 272±51 * | 98±64 | 12±17 |
| Vehicle | 100±11% | 100±15% | 100±23% | 100±33% | 100±23% | 100±44% | 100±9% | 100±22% | 100±22.4% | 100±13% | 100±19% |
| CDAP | | | | | | | | | | | |
| 5 | 146±22% | 77±23% | 92±22% | 244±62% | 3113±1078% * | 2135±592% * | 3619±895% * | 1368±908% | 2935±1205% | 3419±1813% | 2044±915% |
| 10 | 195±45% | 77±14% | 102±17% | 173±42% | 2940±931% * | 1825±524% | 3048±818% * | 3176±1828% | 2604±1005% | 4606±1870% | 1823±1298% |
| 20 | 269±54% * | 52±11% | 146±33% | 208±62% | 1742±1002% | 2187±541% * | 3502±672% * | 7021±2296% * | 4254±794% * | 2444±1595% | 192±109% |

* $P<0.05$ compared to vehicle control, Dunnett's test.

component. After training, animals were returned to the home cage and given access to water for 30 min. For the second and third drug tests for each group, water was withheld for 24 h before the training session. Animals were re-trained for 1 day. After training, animals were returned to the home cage and given access to water for 30 min.

### 2.4. Testing

On day 3 or day 2 (second and third drug tests for a given group of rats), animals were weighed and injected with either

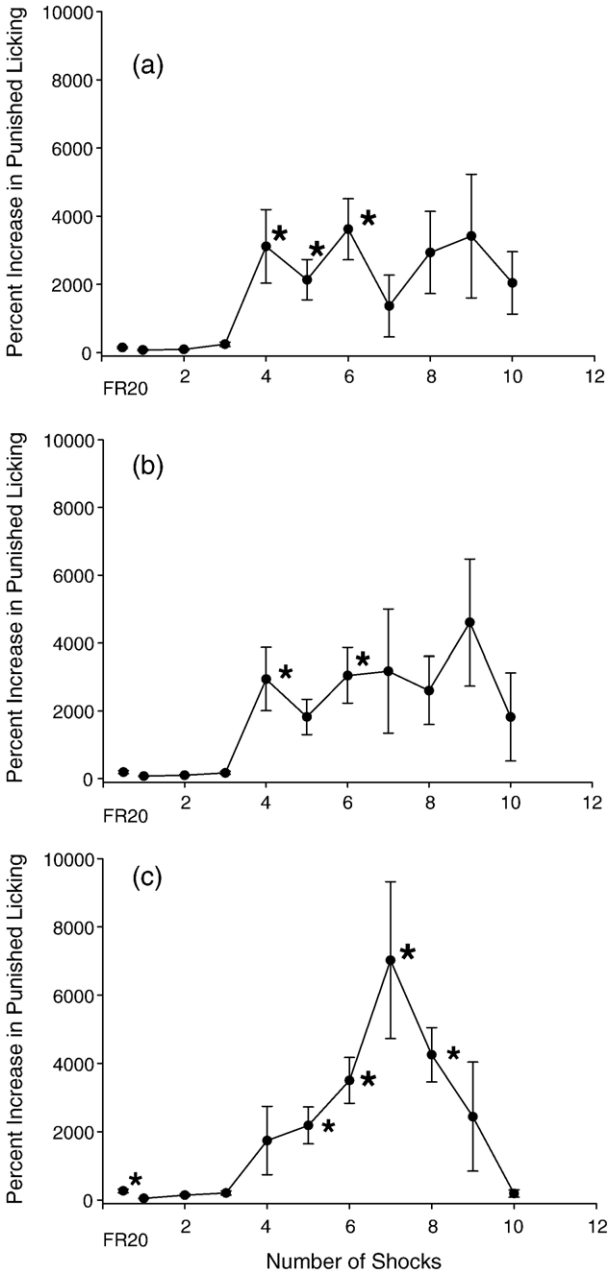


Fig. 2. Effects of a) 5, b) 10, or c) 20 mg/kg chlordiazepoxide on percent punished licking as a function of the number of shocks delivered. Each point represents the mean, and vertical lines are the S.E.M. ($n$=6-8 for each point). *Significantly different than respective vehicle control, $P<0.05$ using ANOVA followed by Dunnett's test.

vehicle or chlordiazepoxide (i.p.) and returned to the home cage (all animals in a cage were injected with the same dose of drug). Thirty minutes after injection, animals were placed into the test chamber. The session was identical to the training session except during the punishment component the sipper tube delivered a brief electrical shock (100 ms, 0.5 mA) after every 20 th lick (FR20) or after 1, several, or all of the first 10 responses (see Table 1 for details of the different conditions that were independently implemented).

### 2.5. Data analysis

The mean number of licks for both the unpunished and punished components was analyzed. In addition, data were also expressed as a percent of control values. The calculation was done using the mean number of licks for the control group in both components. Individual animal means (percent control) were calculated for animals receiving drug utilizing the formula: number of licks divided by mean number of licks by control group times 100 for each respective component. Dose–effect functions were analyzed by ANOVA followed by post-hoc Dunnett's test with vehicle treatment as the control standard. The proportion of animals exhibiting specified numbers of responses was analyzed by Fisher's exact probability test comparing vehicle control to drug values. Statistical probabilities $\leq 0.05$ were considered significant.

### 2.6. Drug

Chlordiazepoxide HCl (Sigma Chemical Co, St. Louis, MO, USA) was dissolved in deionized water and administered i.p. in a volume of 1 ml/kg 30 min pre-session. Drug doses refer to the salt form of the drug.

## 3. Results

CDAP (5–20 mg/kg, i.p.) produced a dose-related increase in punished licking in the standard FR20 procedure (Fig. 1) with a significant increase at 20 mg/kg and no effect on unpunished drinking.

Data for the various shock conditions are presented in Table 2 as both raw numbers of licks and as a percentage of vehicle control values. Percent punished licking data for each chlordiazepoxide dose across shock conditions are presented as separate panels in Fig. 2. In addition, the number of animals making either 10 or fewer licks and 100 or more licks is presented in Table 3. The data, presented in this manner, demonstrates the variation of responding observed across individual animals.

Vehicle control rates of responding were markedly affected by the number and distribution of shocks delivered. As shown in Table 2, there was a 4-fold increase in rates of responding when only the first response during the punishment component was shocked compared to when every 20 responses produced shock. In addition, there was a monotonic decease in control responding when 2, 3 or 4 responses were shocked. Subsequently, increasing the number of shocks delivered did not systematically alter the low rates of responding during the punishment component beyond that already observed under the 4 shock condition. Similar trends in this regard are seen in the quantal measures expressed in Table 3 but dissociations are also apparent mostly within the range of 3–5 shocks. For example, punished response rates were not substantially different from one another and yet the proportion of rats exhibiting 10 or less responses or 100 or more responses varied (compare Tables 2 and 3).

Rates of non-punished responding, which occurred prior to the punishment component, were consistently high: mean ± SD = 447 ± 183 licks within the 3 min unpunished component, giving a rate of approximately 149 licks/min.

Changing the schedule of shock delivery produced both quantitative and qualitative changes in the effects of chlordiazepoxide (Fig. 2). The effects of chlordiazepoxide at 5, 10, and 20 mg/kg varied as a function of the number of shocks delivered (Fig. 2). Chlordiazepoxide (5 mg/kg) significantly increased punished responding only under the 4, 5, and 6 shock conditions and at 10 mg/kg significant increases were seen only under the 4 and 6 shock conditions (Fig. 2a and b). For 20 mg/kg chlordiazepoxide, increases were observed across a wider range of shock conditions and reached, at peak, a greater overall magnitude of effect than at the lower doses.

Table 3
Proportion of rats exhibiting either ≤10 (top panels) or ≥100 licks (bottom panels) during the punishment component

| Treatment | FR20 | 1 shock | 2 shocks | 3 shocks | 4 shocks | 5 shocks | 6 shocks | 7 shocks | 8 shocks | 9 shocks | 10 shocks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Proportion of animals making 10 or less licks* | | | | | | | | | | | |
| Vehicle | 0/6 | 0/8 | 0/6 | 3/7 | 4/7 | 4/6 | 7/7$^{\#\#}$ | 4/4$^{\#\#}$ | 5/5$^{\#\#}$ | 7/7$^{\#\#}$ | 5/6$^{\#\#}$ |
| CDAP | | | | | | | | | | | |
| 5 | 0/6 | 0/8 | 0/8 | 0/8 | 1/8 | 2/8 | 1/8 | 4/8 | 3/8 | 5/8 | 4/8 |
| 10 | 0/6 | 0/8 | 8/8** | 0/8 | 1/8 | 1/8 | 1/8 | 5/8 | 2/8 | 3/8* | 6/8 |
| 20 | 0/6 | 0/8 | 0/7 | 0/7 | 4/8 | 0/8* | 0/8** | 0/4* | 0/8** | 3/8* | 7/8 |
| *Proportion of animals making 100 or more licks* | | | | | | | | | | | |
| Vehicle | 4/6 | 8/8 | 4/6 | 4/7 | 0/7 | 0/6 | 0/7 | 0/4 | 0/5 | 0/7 | 0/6 |
| CDAP | | | | | | | | | | | |
| 5 | 5/6 | 7/8 | 5/8 | 6/8 | 4/8 | 6/8** | 6/8** | 2/8 | 4/8 | 3/8 | 4/8 |
| 10 | 3/6 | 7/8 | 8/8 | 6/8 | 5/8* | 5/8 | 5/8* | 3/8 | 5/8 | 5/8* | 2/8 |
| 20 | 6/6 | 7/8 | 7/7 | 6/7 | 3/8 | 6/8** | 7/8** | 3/4 | 7/8** | 5/8* | 0/8 |

*$P<0.05$; **$P<0.01$ compared to vehicle control within each separate shock frequency condition by Fisher's exact probability test.
$^{\#}P<0.05$; $^{\#\#}P<0.01$ compared to vehicle control of the FR20 condition by Fisher's exact probability test.

The dose-dependence of the effects of chlordiazepoxide was also a function of the number of shocks presented. Although there was a trend for a dose-dependent increase during the punishment component under the FR20 schedule, significant increases were detected only at 20 mg/kg (Fig. 1, Table 1). When 3 responses produced electric shock, chlordiazepoxide did not significantly increase responding. However, when 4, 5, 6, 7, or 8 responses produced shock, increases in responding were observed. These increases were not dose-dependent when either 4, 5, or 6 responses were punished and, under these conditions, 5 mg/kg chlordiazepoxide was able to significantly increase rates of responding. In contrast, under the condition of 7 or 8 responses producing shock, the effects of chlordiazepoxide demonstrated dose-dependence but with only the 20 mg/kg dose significantly increasing rates above vehicle levels.

The maximal increases in suppressed responding were observed under the 7 shock condition in which increases of 7000% of vehicle control values were achieved (in contrast to 270% under FR20 shock). In contrast, no significant increases in responding were observed when either 9 or 10 shocks were delivered. Similar trends in drug effects were seen in the quantal measures (Table 3) although again, these data illustrate variations among individual animals. For example, under the 7 shock condition, half of the animals displayed ten or less responses during the punishment component after 5 mg/kg chlordiazepoxide; two of the rats in contrast displayed 100 or more licks.

## 4. Discussion

The data presented here are the first published, to our knowledge, documenting changes in the effects of the 1,4-benzodiazepine anxiolytic chlordiazepoxide under the Vogel conflict procedure resulting from alterations in the number and distribution of shocks delivered contingent upon licking. Although the current data utilizing an FR20 shock procedure (see Fig. 1) are in agreement with previous reports demonstrating the efficacy of chlordiazepoxide under the Vogel conflict test (e.g. Barrett and Gleeson, 1991), modifications in the effects of chlordiazepoxide under parametric variation were both qualitative and quantitative (see Fig. 2). Manipulations in shock frequency sometimes of only one shock, resulted in modifications in effects of chlordiazepoxide from no effect across doses of 5–20 mg/kg to increases of 7000% of vehicle control levels (Fig. 2, panel c). In addition, changes in the efficacy of chlordiazepoxide were also produced by changes in response-dependent shock deliveries. For example, under the FR20 shock condition, the minimal effective dose for producing increases in licking was 20 mg/kg. When the contingencies of shock delivery were such that some of the first six responses produced shock, the minimal effect dose was at least as low as 5 mg/kg.

Modulation of shock frequency not only altered effects of chlordiazepoxide, but produced profound alterations in the rates of responding observed under vehicle control conditions. As previously demonstrated, response rate can be a major determinant of the effects of drugs on suppressed responding (McMillan, 1973; Witkin and Katz, 1990), it is important to examine the contribution of control rate to the effects of chlordiazepoxide. When only 1, 2, or 3 responses were shocked, control rates of responding were relatively high and chlordiazepoxide did not significantly impact responding. In contrast, control rates of responding were substantially lower when 4 or more shocks were scheduled. Significant increases in responding relative to vehicle values were observed under many of these conditions but not all. Notably, although control responding was comparable when 4 or more shocks were programmed, chlordiazepoxide did not significantly increase responding when the number of delivered shocks was 9 or 10. Additional quantitative changes in effects of chlordiazepoxide were also observed across these conditions of invariant control response rates. Thus, the control rate of response suppression is only one determinant of the behavioral effects of chlordiazepoxide under these conditions. The frequency of shock delivery is independently a powerful controlling variable.

Interestingly, the standard FR20 procedure and the 4 shock paradigm produced distinct effects on both rates of responding under no drug conditions and differential effects of chlordiazepoxide. In both paradigms, animals generally received the same number of shocks (four) in the absence of drug although they were administered in a different temporal sequence. The effectiveness of chlordiazepoxide to increase punished licking was significantly different between the two schedules of shock delivery. In the FR20 paradigm, 20 mg/kg of chlordiazepoxide was the only dose to produce significant increases in punished licking, yet in the 4 shock paradigm, both 5 and 10 mg/kg produced significant increases while 20 mg/kg was without significant effect. The fact that the FR20 procedure and the 4 shock procedure both resulted in comparable numbers of shock indicates that variation in the schedule of shock delivery is an important determinant of behavior and the behavioral effects of chlordiazepoxide, a phenomenon for which there has been ample demonstration under other contexts and with other compounds (see for example, Jeffery and Barrett, 1979; Barrett, 1981; McMillan, 1975). These data also suggest that a host of alternative variations on the Vogel procedure are still unexplored both from the perspective of maximizing the signal detection window, maximizing drug effects, as well as for basic exploration of the variables of which drug effects under this procedure are a function.

Although direct comparisons of the Vogel conflict procedure and the Geller–Seifter conflict procedure have not been made, the effects of drugs across these procedures have generally been comparable (Millan and Brocco, 2003; Cook and Sepinwall, 1975); that is, compounds that increase response rates under one procedure generally increase rates under the other. Likewise, compounds that do not increase responding under the Vogel test also generally do not increase responding under conditions of the Geller–Seifter conflict test. The variables affecting responding in the Geller–Seifter model have been much more extensively studied than under the Vogel procedure (see McMillan, 1975; Witkin and Katz, 1990). In this regard, it is compelling that both shock intensity and shock frequency alter drug effects under the Vogel conflict test as well as the Geller–Seifter conflict test (McMillan, 1975; Meneses and Hong, 1993; Witkin and Barrett, 1976; present study).

The results of the present study, in which the number and distribution of shocks delivered contingent upon drinking,

modified the effects of a known anxiolytic agent, suggest that alterations in shock delivery may be able to modify the potency and magnitude of drug-induced increases in suppressed responding that may predict human anxiolytic efficacy. These different conditions could be exploited to help differentiate different anxiolytic mechanisms. Although untested, we speculate that conditions capable of altering the detection threshold for the 1,4-benzodiazpine anxiolytics, may be of use in defining the range of anxiolytic efficacy of novel compounds in human anxiety states. For example, an experimental condition that yields only small increases in punished responding with chlordiazepoxide might not detect the effects of a novel mechanism whereas conditions which optimize potency and efficacy of chlordiazepoxide might more readily detect other non GABAergic anxiolytic mechanisms.

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