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Antipunishment effects of diazepam on two levels of suppression of schedule-induced drinking in rats

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Abstract

Food-deprived Wistar rats were exposed to a fixed-time (FT) 60-s food delivery schedule until they developed schedule-induced drinking. Rats were matched in pairs according to their licking rates and were designated master or yoked at random. Every fifth lick by master rats was followed by an electric shock during two signalled 5-min periods, which ran concurrently with the food delivery schedule. For the master rats, shock intensities were adjusted to reduce licking to 5–30% (low suppression) or 50–75% (high suppression) of the unpunished licking rates. Yoked rats received the same shocks as master rats, but independently of their own licking. The drinking by yoked animals was not decreased by the presentation of these lick-independent shocks. Diazepam (0.3–10.0 mg/kg) was studied for its effects on punished and nonpunished schedule-induced drinking. Intermediate doses of the drug increased the punished behavior of master rats, but only when schedule-induced drinking was highly suppressed. Diazepam dose dependently decreased licking rates in all other conditions. The antipunishment effects of benzodiazepines may depend on the level of suppression of schedule-induced drinking, and this is in keeping with the results of other experimental preparations where behavior was under aversive control.

Keywords: Schedule-induced drinking; Adjunctive behavior; Punishment; Response-independent shock; Diazepam; Lick; Rats

1. Introduction

Geller et al. [8–10] were the first to demonstrate that barbiturates and benzodiazepines increase punished operant behavior. This effect has been replicated with different schedules of reinforcement and punishment, as well as with different animal species (see Refs. [11,15] for reviews).

Houser [11] considered various possible mechanisms for the anticonflict effect of anxiolytics. He emphasized that changes in the parameters of the punishment schedule, such as the shock intensity or its frequency, are important determinants of the effect of anxiolytic drugs. For example, it has been reported that diazepam had only a small tendency to increase punished behavior when response rates were not very markedly suppressed [14]. However, when response rates were highly suppressed by a larger shock intensity, diazepam increased punished behavior to a greater extent. The changes in the parameters of the punishment schedules determined the degree of suppression of operant behavior [11]. Therefore, the degree of suppression of the punished behavior might be an important mechanism to explain drug effects on response rates reduced by punishment procedures.

Cook and Catania [2] studied the possibility that the effects of anxiolytics could be explained in terms of basal response rates, because response rates normally are lower on punishment than on nonpunishment conditions. They exposed squirrel monkeys to a conjoint variable-interval (VI) 6-min VI 2-min food reinforcement schedule. Both VI schedules run simultaneously associated with the same response lever, but food could be obtained just in one component at a time. Alternation between components was possible through a changeover response on a different lever. A VI 2-min shock schedule was then superimposed on the VI 2-min food schedule, which produced a punished response rate similar to that maintained by the unpunished VI 6-min component. Meprobramate and chlordiazepoxide produced a greater increase of punished than of unpunished responding. This result suggests a specific effect of anxiolytic drugs on punished behavior, which was not simply

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determined by the low basal response rates. Similar outcomes have been obtained with other reinforcement schedules and with other benzodiazepines and barbiturates [3,13,16,23] (however, see Refs. [24,26]).

The effects of chlordizepoxide recently have been reported to depend on the basal rate of suppressed responding [4]. Pigeons were exposed to a random-ratio schedule of food reinforcement. The rate of pecking was then reduced to different levels by the presentation of two stimuli correlated with electric shocks of different intensities or by the omission of food (extinction). The drug produced greater increases in the response rates that were the more suppressed, which suggested a contribution of response rate to the effect of the drug on responding that has been reduced by the response-independent presentation of aversive events.

Food-deprived rats exposed to intermittent food reinforcement schedules typically drink water after each food pellet is delivered, resulting in excessive water intake [5]. It has been suggested that this behavior is an example of a more general class of behavior different from operant and respondent behaviors [6]. The nature of adjunctive behavior, however, remains to be elucidated (for reviews, see Refs. [18,19,21,25]). In contrast to the vast literature on the effects of drugs on punished schedule-maintained behavior, there are very few studies in which the antipunishment effects of drugs on schedule-induced behavior have been assessed.

Hymowitz [12] exposed rats to a multiple fixed-interval (FI) 40-s FI 40-s schedule of food reinforcement; the components were signalled by the houselight. A multiple variable-time (VT) 125-s VT 125-s schedule of shock presentation was then superimposed on the food schedule. Each shock delivery in the illuminated component was signalled by a 5-s white noise (signalled shocks), and the shock was presented at the time the noise ended. During the nonilluminated component, shocks were unsignalled. Responses during the 5-s preshock periods were not included in the calculation of overall response rates. Diazepam produced a dose-dependent increase in the overall suppressed rates of lever pressing and schedule-induced drinking during signalled and unsignalled shocks (differential suppression), but had little effect on either behavior when they were reduced by the presence of the white noise (conditioned suppression). Increases after diazepam were greater as shock intensity increased and when shocks were signalled.

Pellón et al. [20] studied the effects of diazepam on schedule-induced drinking punished with lick-contingent delays in the administration of food pellets. Their results indicated that doses of diazepam between 0.5 and 4.0 mg/kg did not increase punished schedule-induced polydipsia. D-Amphetamine did increase the reduced rates of schedule-induced drinking, however. The results of Pellón et al. are different from those generally found with operant behavior. The punishment procedure was a negative contingency between drinking and food presentation, which is different from the positive contingency between behavior and shock delivery normally employed in operant studies.

Flores and Pellón [7] exposed rats to a fixed-time (FT) 60-s food schedule until they developed schedule-induced drinking. Rats were matched in pairs according to their licking rates, being designated master or yoked at random. Every fifth lick by master rats was then followed by an electric shock (0.05, 0.1, or 0.2 mA), while the food schedule continued in operation. Yoked rats received the same shocks, but independently of their own licking. Diazepam (0.5 to 2.0 mg/kg), but not D-amphetamine, increased punished schedule-induced drinking, and this effect was greatest at the 0.1-mA shock intensity. Different shock intensities normally produce reductions that are proportional to the intensity of the shock, prompting the need to investigate the role of different degrees of suppression on the effect of anxiolytic drugs. This is the aim of the present experiment.

The high rates of licking a water spout, induced by a FT 60-s schedule of food delivery, were punished by the presentation of lick-contingent shocks during signalled 5-min periods. Shock intensities were adjusted to produce a high suppression of licking in half of the master rats and low suppression in the other half. Diazepam effects were then tested on punished and nonpunished schedule-induced drinking. The experiment included yoked rats, which received the same shocks as the master animals but independently of their own licking, in order to compare the effects of the drug on the behavior maintained by response-dependent and response-independent shocks.

2. Materials and methods

2.1. Subjects

The subjects were 12 experimentally naive male Wistar albino rats, obtained from IFFA-CREDO (Lyon, France). They were 90 days old at the start of the experiment, with a mean free-feeding body weight of 414 g (range: 380–432 g). The rats were housed individually in an environmentally controlled room (22°C temperature, 60% relative humidity, and 08:00/20:00 hours light/dark cycle) in the Animal Laboratory of the Facultad de Psicología, Universidad Nacional de Educación a Distancia. After a period of 10 days of habituation to the housing conditions, and before training, the rats were gradually reduced to 80% of their free-feeding weights by controlled feeding. Each rat was maintained at that weight; it was weighed before each experimental session, and at least 15 min after the session, it was given an appropriate supplement to the food it had obtained in the experiment Water was continuously available in the home cages. All animal use procedures were in accordance with the European Communities Council Directive.

2.2. Apparatus

The experiment was conducted in six identical Letica Instruments LI-836 (Barcelona, Spain) test chambers, 29 cm long × 24.7 cm wide × 35.5 cm high, with grid floors. Each chamber was contained inside a ventilated sound-attenuating chest, with a small observation window in the left wall. The intelligence panel of the test chamber was aluminum, the right-side wall was dark acrylic, and the other two sides and the roof were transparent acrylic Operant levers were withdrawn during the course of the experiment. Six Letica LI 100-20 generators supplied scrambled electric shock individually through the grid floor of each box. A calibrated water bottle was mounted on the outside of the right wall of each chamber, with its spout accessible to the rat through a hole 3.2 cm wide × 3.9 cm high, situated 20 cm from the front wall and 7 cm above the grid floor. The spout was positioned 2 cm behind the hole, so that the rat could lick it but could not maintain permanent contact with it. Licks at the spout created a circuit closure between the bottle spout and the grid floor, and generated a pulse. The spout was not included in the shock circuitry, and the recording of licks was not interrupted by shock delivery. Two 3-W houselights illuminated each test chamber during experimental sessions. The ambient noise produced by the ventilation fan was 60 dB, which served as masking noise. A Letica Instruments pellet dispenser was located behind each front panel and delivered 45-mg pellets of standard rat food (Bio-Serv) to a receptacle in the center of the front wall of the chamber, situated 3.7 cm from the grid floor. The scheduling and recording of experimental events was achieved by means of a BBC microcomputer (Acorn Computers) programmed in SPIDER.

2.3. Procedure

When each rat had stabilized at 80% of its free-feeding weight, a water-ingestion test was given on 2 successive days. Fifty-five 45-mg food pellets were placed together in a dish in the home cages, and the amount of water consumed by each rat in 55 min was measured. This measure provided a baseline against which to assess the degree of any schedule-induced polydipsia subsequently observed in the experiment, in which each animal received individually over a period of 55 min a number of pellets identical to that given during the water-ingestion test.

On the next day, the rats were adapted to the test chambers for 55 min, and they were allowed to eat 20 food pellets that previously had been placed in the food receptacles. The water bottles were not installed.

After this feeder training, rats were exposed to a FT 60-s schedule of food pellet presentation during 55-min sessions. The food pellets were delivered at regular 1-min intervals, regardless of the animal's behavior. The bottles were filled with 100 ml of fresh tap water and were installed in the boxes immediately before each experimental session. Each session began with the illumination of the boxes. Sessions were conducted 5 days a week. The following measures were recorded for each rat in each session: (a) the total number of licks, which allowed the calculation of the number of licks per minute; and (b) the total amount of water (ml) removed from the bottle.

After 28 sessions, when the data revealed no systematic within-subject variation, rats were paired according to their licking rates. For each pair of rats, one was randomly assigned as a master rat and the other as a yoked rat. A multiple schedule of food reinforcement then was introduced. Food pellets continued to be delivered at 60-s intervals during 55-min sessions. Two 5-min periods signalled by a tone (70 dB, 40 Hz) were then superimposed on the food schedule. These signalled periods were initiated after 15 and 35 min from the start of each experimental session, respectively. Every fifth lick made by a master rat within these periods was followed by a 0.3-s electric shock to that rat through the grid floor (a fixed-ratio five-shock schedule). The intensity of electric shock was adjusted for each master rat to obtain a certain degree of suppression in the rate of licking in comparison to unpunished licking. The licking of half of the master animals (rats 1, 3, and 5) was reduced to between 5% and 30% of the licking recorded during the unpunished component. These rats were labeled "low suppression," resulting that the range of the intensities of electric shocks was between 0.05 and 0.07 mA. The licking of the other half of master animals (rats 7, 9, and 11) was reduced to about 50–75% of the rate of licking during the unpunished component, and as a consequence, they received electric shocks of intensities between 0.10 and 0.12 mA. These rats therefore belonged to the condition of "high suppression." Yoked rats received the same number and the same intensity of electric shocks as their respective master rats, but shocks were not programmed to occur contingent on their own licking. Licks were recorded independently during the shock and no-shock components for each rat, which allowed the calculation of the number of licks per minute for each component. Overall water consumption also continued to be recorded, but it will be not reported because the intake could not be differentiated for each component independently. This experimental stage lasted 30 sessions, except for rats 9 and 10, which lasted 60 sessions.

Each rat was then exposed to the administration of diazepam at doses of 0.3, 1.0, 3.0, and 10.0 mg/kg (rats 9 and 10 were not given the highest dose). The drug was suspended in a solution composed of distilled water and three drops of Tween 80 (Sigma-Química, Madrid, Spain), and was administered by intraperitoneal injection in a volume of 1 ml/kg body weight 10 min before testing. Drug doses were given in a random order, and there were two sequences of independent randomized doses. All rats received the same dose on the same day, and the yoking procedure remained in operation during drug sessions. Drug sessions were on Tuesdays and Fridays. On Thursdays,

animals received vehicle administrations in a volume of 1 ml/kg. The sessions ran on Mondays served as control condition without injection. The pharmacological study encompassed 20 sessions, and licking rates were still calculated independently for the shocked and the nonshocked components.

3. Results

All rats developed schedule-induced polydipsia after being exposed to the FT 60-s schedule of food presentation. During the last 5 days of the first phase of the experiment, rats drank on average about 28 ml (range: 19–42 ml), which was more than four times higher than the consumption of water during the home cage ingestion test (6.5 ml on average, range: 5.0–8.5 ml). With the introduction of the conflict procedure, the licking rates of master rats were reduced in the shocked component to between 5% and 30% for the lower suppression animals, and to between 50% and 75% for the higher suppression animals, with respect to their licking rates during the nonshocked component.

Fig. 1 shows the effects of the conflict procedure on the rates of licking of master and yoked rats for both shocked and nonshocked components. Each panel represents a pair of master and yoked rats. The rate of licking was lower during shocked (closed circles) than nonshocked (open circles) components for all master rats, as can be seen by the data above control sessions (C). This difference was smaller for master rats 1, 3, and 5 (left-hand panels) than for rats 7, 9, and 11 (right-hand panels), which is a reflection of the different levels of suppression reached by the two experimental treatments. In the case of rat 1, unpunished licking was also affected by the introduction of the shock treatment. Licks per minute of yoked rats in the shocked (closed triangles) and the nonshocked (open triangles) components do not reflect similar changes to those seen with master rats. With the exception of rat 6, which showed a reduction similar to that of its master rat during the shocked component, and of rat 10, for which licking was completely suppressed during the shocked component, all other yoked rats showed rates of licking that were comparable in both components. Even more, at times, licking was higher in the shocked component (see rat 8).

Fig. 1 also shows the effects of diazepam on the rates of schedule-induced drinking. Except for rat 3 in the nonshocked component, the administration of vehicle (V) did not have any significant effect on the licks per minute of both shocked and nonshocked components for any of the subjects in comparison with the licking rates when no injection was given. Generally, diazepam did not increase the punished or unpunished rates of licking of master rats submitted to a lower suppression (left-hand panels). A small increase was observed in the rates of punished and unpunished licking of master rat 5 at the dose of 0.3 mg/kg, and at the dose of 1.0 mg/kg in the licking of master rat 1 during the unpunished component. As the dose of diazepam increased, dose-dependent reductions in the licking rate of master rats both in the shocked and the nonshocked components can be observed. The dose of 1.0 mg/kg reduced the rate of licking of master rat 5 in the nonshocked component, and the doses of 3.0 and 10.0 mg/kg almost completely abolished schedule-induced drinking in all master animals.

Diazepam also dose dependently reduced the rates of licking of yoked rats belonging to the low suppression treatment, both during the shocked and the nonshocked components. The doses of 0.3 and 1.0 mg/kg did not generally alter the licking rates in comparison with control conditions. Licks per minute were reduced by the dose of 3.0 mg/kg, and the dose of 10.0 mg/kg almost completely suppressed schedule-induced drinking. Diazepam, however, increased at times the schedule-induced drinking of these yoked animals. The licks per minute of rat 6 during the shocked component were markedly increased after 1.0 mg/kg, and a small increase can also be observed in the licking rate of rat 2 during the nonshocked component when given the 0.3 mg/kg dose.

The punished rates of licking of master animals submitted to a higher suppression were increased by different doses of diazepam (Fig. 1, right-hand panels). The doses of 0.3 and 1.0 mg/kg increased the licking of all master rats, and even the dose of 3.0 mg/kg increased licks per minute in rats 7 and 11. This dose, however, completely abolished the drinking of rat 9. Schedule-induced drinking was also totally eliminated after the administration of the 10.0 mg/kg dose in the other master rats. The unpunished drinking was marginally increased in master rat 9 at the dose of 0.3 mg/kg. In general, however, diazepam at 0.3 and 1.0 mg/kg did not alter the rates of licking of master rats during the unpunished component.

Diazepam dose dependently reduced the rates of licking for the yoked animals of the higher suppression condition. The doses between 0.3 and 3.0 mg/kg, generally, did not change the licks per minute of these animals. However, small increases were observed in the licking of rat 8 during the nonshocked component, and in the licking of rat 12 during the shocked and nonshocked components, after the administration of 1.0 mg/kg. Licks per minute were completely abolished at the dose of 3.0 mg/kg in rat 10. The dose of 10.0 mg/kg resulted in a complete cessation of licking in the remaining yoked rats.

Fig. 2 represents the effects of diazepam on the rates of licking of master rats during the shocked component as a function of the level of suppression of schedule-induced drinking. The ordinate axes denote the logarithm of the rate of licking after the administration of diazepam, calculated as a proportion of the rate of licking in control sessions. The abscissa is the inverse of the suppression of behavior maintained during control conditions. The values on the left of this axis represent low rates of licking, and the

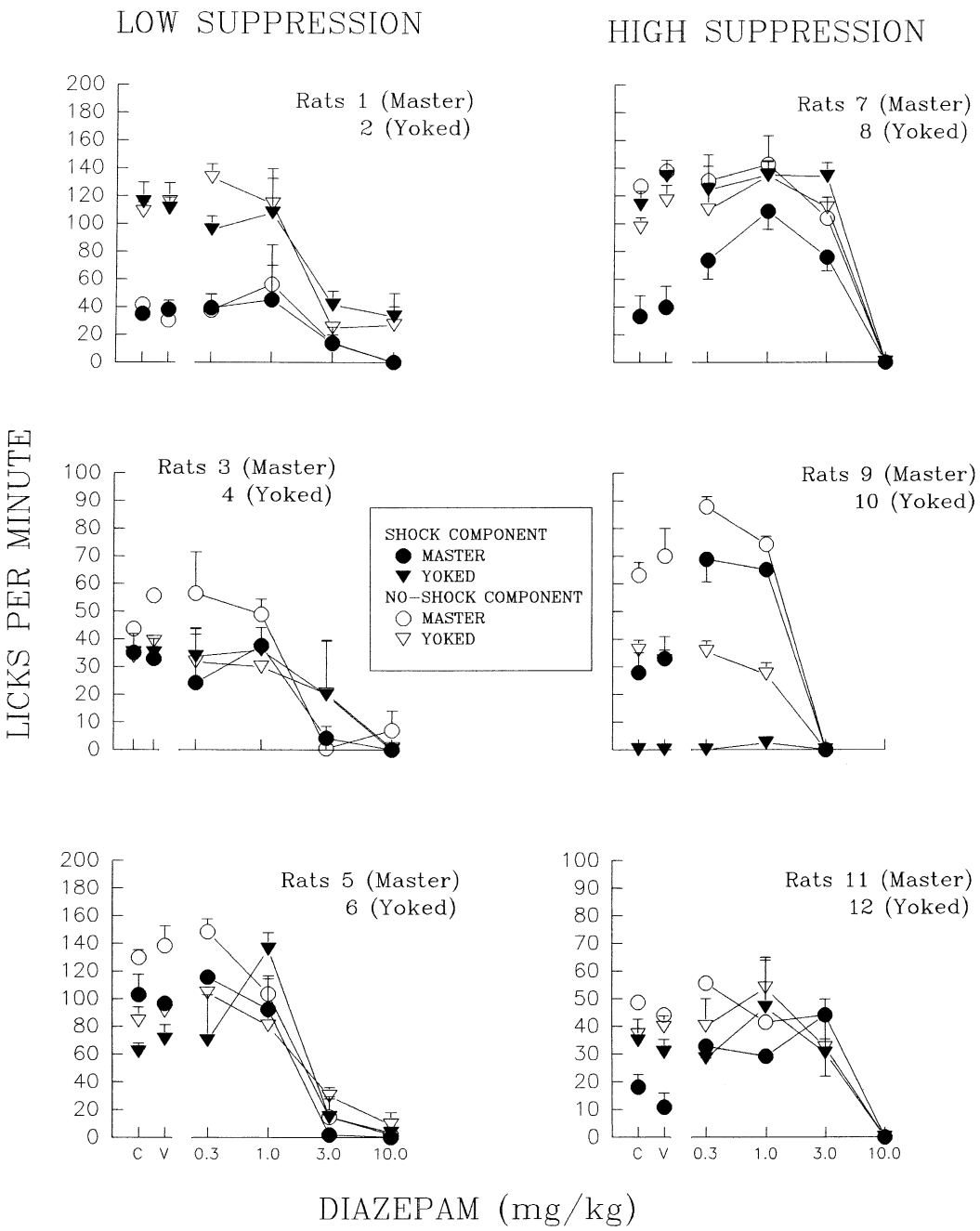

Fig. 1. Effects of diazepam on the rates of licking induced by a multiple FT 60-s schedule of food pellet presentation, with components in which licks were unpunished or punished by shock delivery (see text). The data for each dose of diazepam are the means of two administrations for each dose. Control data (C) are the means of four sessions in which animals received no injection (three sessions for rats 9 and 10). Vehicle data (V) are the means of four sessions of vehicle administration (three sessions for rats 9 and 10). Filled and open circles represent the rates of licking of master rats during shocked and nonshocked components, respectively. Filled and open triangles represent the rates of licking of yoked animals during shocked and nonshocked components, respectively.

values on the right denote high rates of licking, which correspond to the licks per minute maintained by master rats exposed to a high or a low suppression, respectively. Regression lines were fitted to the data using the method of least squares.

At the doses of 0.3, 1.0, and 3.0 mg/kg (excluding 3.0 mg/kg for rats 9 and 10), there was a trend for the lower rates of licking to be increased more by diazepam than higher rates. All doses produced increases in the highly suppressed response rates and decreases in the response rates that were less suppressed; however, this latter effect was more marked at the dose of 3.0 mg/kg. The Pearson's correlation and the linear regression lines were: $r=-0.84$, $y=-8.39+0.45x$, for 0.3 mg/kg; $r=-0.79$,

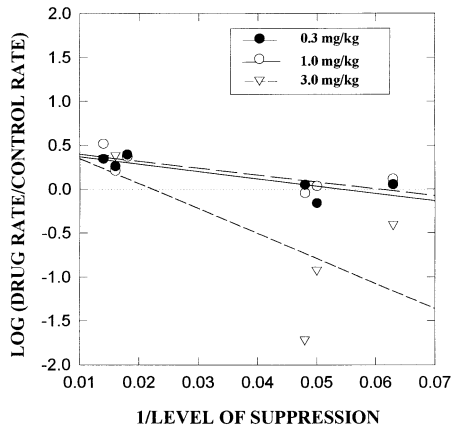

Fig. 2. Effects of diazepam on the rates of licking of master rats during the punished component, expressed as the logarithm of the effect of the drug as a function of the inverse of the level of suppression. The dashed line passing through zero on the vertical axes denotes no effect of the drug; the positive and negative values correspond to increases or decreases in the rates of licking after the administration of diazepam. Each data point is the mean of two observations for each rat at each dose.

$y=-7.88+0.47x$, for 1.0 mg/kg; and $r=-0.70$, $y=-28.45+0.63x$, for 3.0 mg/kg.

4. Discussion

The rates of licking induced by a FT 60-s food schedule were reduced in one of the components of a multiple schedule by means of lick-contingent shock delivery. The intensity of electric shocks was adjusted for each master rat to reduce schedule-induced drinking proportionally low or high in comparison with the levels of licking induced during the nonshocked component of the multiple schedule. Yoked animals received the same electric shocks as their corresponding master rats but independently of their own licking. The schedule-induced drinking of yoked rats was not decreased by the presentation of lick-independent electric shocks. This result shows that schedule-induced polydipsia can be punished at different levels of suppression by the contingent presentation of electric shocks [1,7], and that lick-independent shocks are not sufficient to reduce schedule-induced drinking.

Moderate or intermediate doses of diazepam increased schedule-induced drinking that had been punished, as it was the case with the master rats submitted to a high suppression procedure. These increases were not observed in the unpunished licking for those same master rats nor in their yoked animals. Diazepam also did not generally increase the licking rates of master and yoked rats submitted to a low suppression procedure. Except for master rats of the high suppression condition during the shocked component, diazepam dose dependently decreased the rates of licking. With the master rats of the higher suppression, diazepam produced at the highest dose tested a complete reduction in the rate of punished schedule-induced drinking.

The only notable exception to the above results is that yoked rat 6 increased the licking rate during the shocked component after the 1.0 mg/kg dose of diazepam. This anomalous result can be explained by the higher suppression of licking observed with this rat in comparison to other yoked animals during the shocked component. Yoked rat 10 also suppressed licking during the shocked component; however, this rat did not lick at all and, therefore, the potential effects of diazepam were difficult to observe. Lick-independent shocks can sometimes decrease the rates of schedule-induced licking [7,12]. These suppressed rates of licking can then be increased by diazepam [12] (however, see Ref. [7]).

It is difficult to interpret the effect of diazepam on licking maintained by response-independent shock in the present study, because punished licking of the master animals was affected by diazepam. A control condition describing the effects of diazepam in the licking of yoked animals in the absence of drug delivery to master rats is required before firm conclusions can be drawn regarding the effects of drugs on response suppression due to independent presentation of shocks.

In general, diazepam only increased the low rates of punished schedule-induced drinking, and these increases were dependent on the suppression level of adjunctive drinking. Therefore, the antipunishment effects of diazepam were dependent on the rate of punished drinking, being observed by the increases of the lower rates of licking, and by the decreases of the higher rates. Furthermore, suppression-dependent effects were more marked as the dose of diazepam increased. These results complement and amplify previous results with diazepam on punished schedule-induced drinking and schedule-controlled lever pressing. It has been reported that diazepam exerted a more pronounced antipunishment effect on the rates of operant behavior that were more suppressed [14]. It has been also shown that punished operant behavior was more sensitive to be increased by anxiolytic drugs than operant behavior reduced by response-independent shocks [17]. A similar result has been observed with adjunctive drinking [7].

Diazepam seems to exert antipunishment effects on schedule-induced polydipsia and operant behavior by means of a mechanism that might be called suppression dependency. These effects might be a case, however, of the more general effect of rate dependency (see Ref. [22]). Some authors have observed that diazepam or chlordiazepoxide similarly increased low rates of operant behavior regardless if they were punished or not [24,26]. Other authors have found that benzodiazepines just increased punished operant behavior [2,13,23].

With regard to schedule-induced behavior, it is premature to have a definitive conclusion. The present data were obtained from master and yoked animals that were not matched in their rates of licking during the shocked component. However, several of the results presented here seem to contradict the general idea of the rate-dependency hypothesis. For example, licks per minute of master rat 1 were

much lower than those of yoked rat 2; however, diazepam did not increase the low licking rate of rat 1. Master rats 1 and 3 licked during the shocked component at a similar rate to master rats 7 and 9, but diazepam only increased the licking of these last two rats. If the rate-dependency mechanism were responsible for the observed effects of diazepam, master rat 1 should have increased licking after diazepam both on the shocked and the nonshocked components in comparison with yoked rat 2, and yoked rat 10 should have increased the licking on the nonshocked component similar to master rat 9 on the shocked component.

The antipunishment effects of diazepam on schedule-induced drinking appear to depend on the degree of suppression of the behavior. The rate increases produced by diazepam occurred when the suppression of licking was greater. This does not necessarily mean that the behavioral effects of diazepam cannot be explained according to a general principle of rate dependency. Our results suggest that the rate-dependency principle has limited validity. However, more specifically designed experiments are needed to test the effects of benzodiazepines on punished schedule-induced drinking maintained at rates similar to nonpunished schedule-induced drinking.

The effects of diazepam as suppression dependent are applicable to adjunctive and operant patterns of behavior reduced by different aversive procedures. This is the case for punished operant behavior, as has been shown previously. More recently, similar effects of chlordiazepoxide on operant response rates suppressed by a conditioned suppression procedure [4] have been shown. Even more, it has been found that schedule-induced drinking reduced by lick-independent electric shocks of high intensity was increased by diazepam as a function of the level of suppression [12]. All these data are similar to the results obtained in the present experiment on punished schedule-induced drinking, and in summary, emphasize that the behavioral effects of benzodiazepines are similar in all behaviors under aversive control, being schedule induced or schedule maintained.

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