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Punishment Modifies the Effects of Chlordiazepoxide and Benzodiazepine Receptors

SARI IZENWASSER,[1] MICHAEL J. BLAKE,[2] NICK E. GOEDERS AND STEVEN I. DWORKIN[3]

Departments of Psychiatry and Pharmacology & Therapeutics
Louisiana State University Medical Center, Shreveport, LA

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IZENWASSER, S., M. J. BLAKE, N. E. GOEDERS AND S. I. DWORKIN. *Punishment modifies the effects of chlordiazepoxide and benzodiazepine receptors.* PHARMACOL BIOCHEM BEHAV **32**(3) 743–748, 1989.—Littermate groups of male albino rats responded under a procedure which generated comparable rates of punished and nonpunished responding. Chlordiazepoxide (3.0–30.0 mg/kg, IP) increased punished responding but had no effect on nonpunished responding. Homogenate receptor binding studies with [^{3}H]Ro 15-1788 indicated increased benzodiazepine receptor binding in the striatum of rats who received shock. Moreover, a third group of rats exposed to noncontingent shock showed greater increases than those whose responses had been punished, suggesting that predictability and control of shock may have attenuated the effects of the noxious stimulus. Increased binding seen in the cerebellum, however, was related to the punishing effects of the electric shock since it occurred only in those animals receiving response-contingent shock. There were no changes in binding affinity in any of the brain regions tested. Site-specific alterations in benzodiazepine receptors following electric footshock are related to the contingencies under which the noxious stimuli are administered. Furthermore, changes in benzodiazepine receptor binding may underlie the differential effects of benzodiazepine agonists on punished and nonpunished responding.

Punishment Chlordiazepoxide Receptor binding Benzodiazepines Rats

BENZODIAZEPINES and other widely prescribed anxiolytic agents have been shown to increase punished responding by laboratory animals. For example, chlordiazepoxide and meprobamate will increase responding in rats that was suppressed by electric footshock (5, 8, 9, 17). However, drug-related increases in low rates of behavior not suppressed by punishment have also been reported following the administration of anxiolytic compounds (6,11) suggesting that the changes in response rate may not be punishment-specific but may represent nonspecific rate effects. Therefore, it is important to control for baseline rates of responding in investigations of the punishment-specific effects of pharmacological agents. In the present study, a procedure which generated comparable rates of punished and nonpunished responding was used to investigate the effects of chlordiazepoxide.

Benzodiazepines interact with specific receptors in the central nervous system (3, 15, 20) and there are strong correlations between the binding affinities of benzodiazepine agonists to these receptors and their potencies as anxiolytic agents in humans and anticonflict agents in animals (15). Therefore, it is likely that the behavioral effects of benzodiazepines result from direct interactions with specific receptors. Since any punishment-specific effects of chlordiazepoxide are likely due to interactions with benzodiazepine receptors, the effects of punishment on the binding of [^{3}H]Ro 15-1788 were also investigated.

METHOD

Subjects

Male rats originally derived from the Fischer 344 strain and approximately 90 days old at the beginning of the study were used. The animals were housed in individual cages located in a temperature- and humidity-controlled animal care facility on a reversed 12-hour light/dark cycle (lights off 07.00). The rats were maintained at 80% of their unrestricted feeding weights and had continuous access to water except during experimental sessions.

Apparatus

During the experimental session, the rats were placed in standard operant conditioning chambers constructed of aluminum and Plexiglas. These chambers were located in ventilated, sound-attenuating enclosures in a room with white noise. Three feedback

[1]Present address: Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD.
[2]Present address: National Institute on Aging, Gerontology Research Center, Baltimore, MD.
[3]Requests for reprints should be addressed to Steven I. Dworkin, Ph.D., Department of Psychiatry, LSU School of Medicine, P.O. Box 33932, Shreveport, LA 71130.

TABLE 1

RESPONSE RATES FOR THE RATS ON THE RANDOM-RATIO AND VARIABLE-INTERVAL SCHEDULES BEFORE AND AFTER THE ADDITION OF SHOCK TO THE RATS ON THE RANDOM-RATIO SCHEDULE

	Random-Ratio		Yoked Variable-Interval	
	Preshock	Shock	Preshock	Shock
P1	58.65 ± 15.45	10.92 ± 10.50	21.93 ± 4.66	1.40 ± 0.96
P2	48.98 ± 18.32	4.00 ± 2.72	12.93 ± 5.41	1.13 ± 1.55
P3	97.10 ± 27.57	6.58 ± 4.11	10.65 ± 5.90	0.50 ± 0.32
P4	22.31 ± 2.75	23.22 ± 17.58	5.64 ± 1.49	3.27 ± 2.24
P5	33.31 ± 3.44	3.47 ± 2.99	6.50 ± 3.66	0.55 ± 0.48
P6	66.16 ± 20.76	2.30 ± 1.36	14.48 ± 3.16	1.15 ± 0.78

relays and a transformer (24 V AC) were mounted on the inside ceiling of these enclosures. Sessions were controlled and data collected and analyzed using Rockwell Aim 65 computers operating under MCS control (Micro Interfaces) which were located in an adjacent room. A food receptacle, response lever and a stimulus light (24 V AC) were located on one wall of the experimental chamber. The food cup was connected to a pellet dispenser that delivered 45 mg pellets (BioServe). Each lever press momentarily operated a "feedback" relay and darkened the stimulus light. The floor of the chamber consisted of stainless steel rods placed parallel with the front wall. The rods were connected to a scrambled shock source (Coulbourn Instruments).

Behavioral Procedure

Six littermate pairs of rats (P1–P6) were trained simultaneously on a yoked procedure previously described (7). Responding by one subject of each pair was maintained by a random-ratio (min 5/max 100 responses) schedule of food presentation. The interreinforcement intervals from this subject were used to generate a yoked variable-interval schedule of reinforcement for a littermate. When response rates for rats on both the ratio and yoked variable-interval schedules had stabilized, a conjoint random-ratio schedule (min 25/max 200 responses) of electric footshock (0.6 mA, 100 msec in duration) was added to the random-ratio schedule of food presentation. Under this schedule, the subject on the ratio schedule received response-contingent food and shock presentation, while the rat on the yoked-interval schedule received only response-

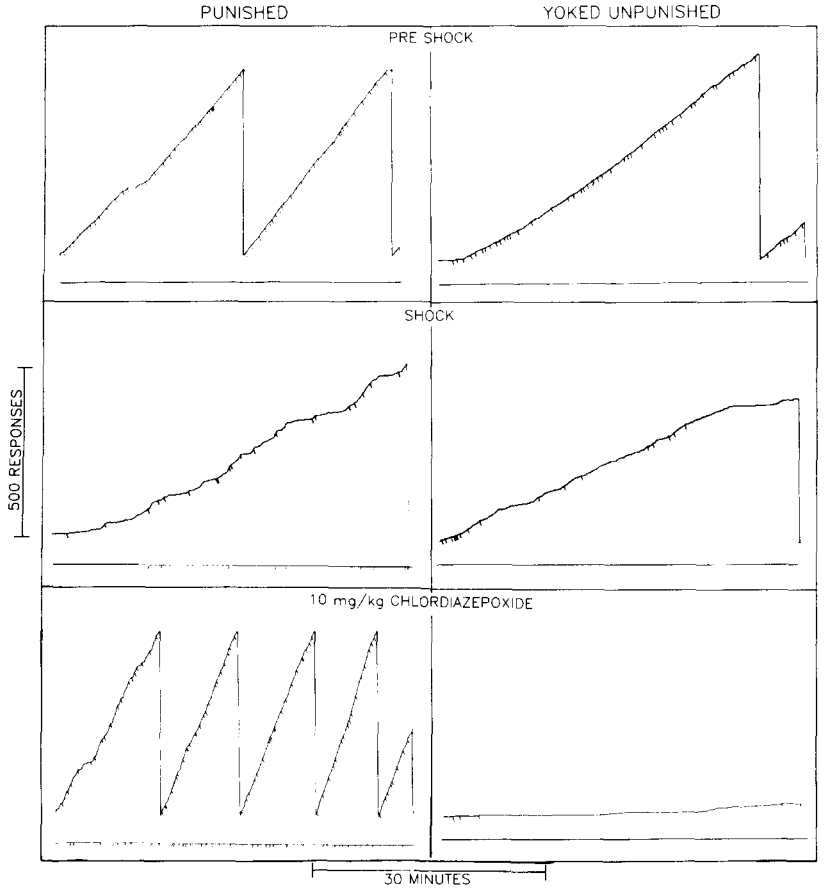

FIG. 1. Representative cumulative response records from a rat on the random ratio (left side) and yoked variable-interval (right side) schedules. The top row depicts responding before the shock contingency was introduced to the rat on the random-ratio schedule. The middle records depict the effects of the shock contingency and the bottom records show the effects of 10.0 mg/kg chlordiazepoxide on each rat's responding. Deflections of the top and bottom pens indicate food presentations and shock deliveries, respectively.

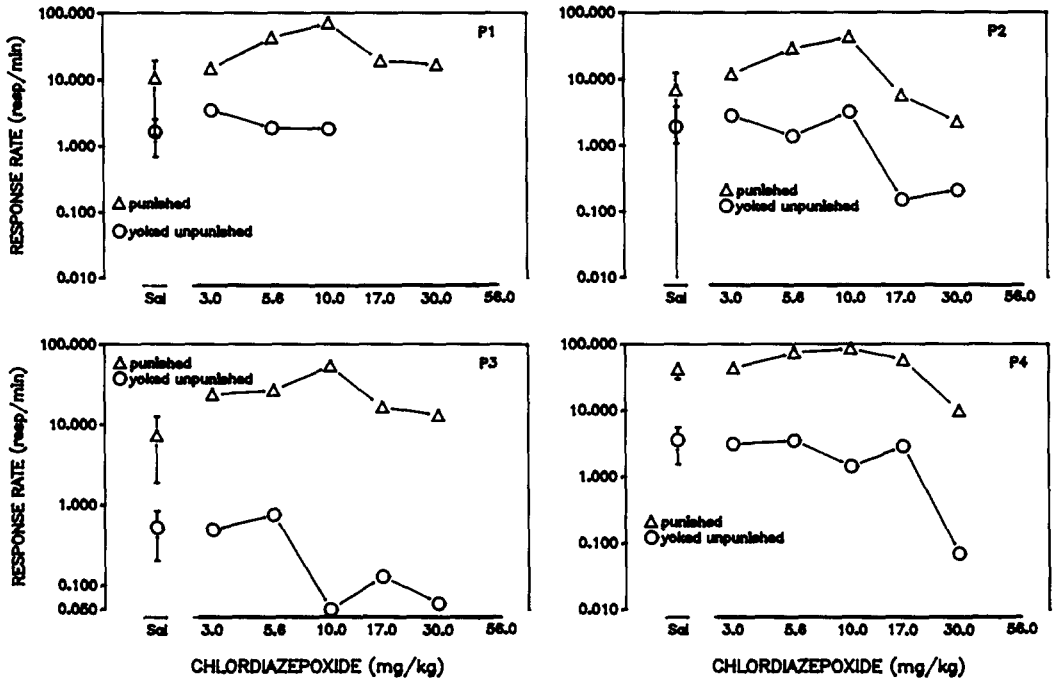

FIG. 2. Dose-response curves for the effects of chlordiazepoxide on response rates maintained by both schedule contingencies for the rats displaying increases in punished responding. The open triangles and circles show the effects of the drug on punished and nonpunished responding, respectively. Both response rate and dose are presented on a log scale.

contingent food presentations. Daily sessions were terminated after 45 min or the delivery of 100 food pellets to the rat on the yoked-interval schedule.

Once responding had stabilized under this schedule, chlordiazepoxide (3.0–56.0 mg/kg, expressed as salt) or saline was administered intraperitoneally in volumes of 1 ml/kg body weight 15 min before the start of the session. Each dose was evaluated twice using two ascending series of doses in each subject. Vehicle days were always interspersed between each day of drug treatment so that animals received drug only twice weekly. Only one subject of each pair received drug on a given day.

Receptor Binding Procedure

Seven littermate triads of rats (T1–T7) were trained simultaneously on the yoked procedure. The first two rats of each group were trained as above and the third animal of each group received noncontingent food and shock presentation yoked to the schedule generated by the rat on the random-ratio schedule. The rats were trained under these conditions for 30 sessions and were then sacrificed by decapitation. The brains were removed and rapidly dissected over ice into frontal cortex (FCX), hippocampus (HIP), striatum (STR), diencephalon (DNC), cerebellum (CBL), and brain stem (BS).

The dissected brain regions were frozen on dry ice and stored at −70°C until assay. Membranes were prepared by tissue homogenization (Polytron, at setting p-10 for 15 sec) and centrifugation (15000 rpm for 10 min) in 15 ml of ice-cold 50 mM tris HCl (pH 7.7) two times, with the supernatant discarded. The final membrane pellet was diluted to 1.7 mg/ml (approx. 150 μg protein/ml). The binding assay consisted of incubating triplicate samples of tissue (1.0 ml) at 4°C for 60 min with [^{3}H]Ro 15-1788 (10^{-9} M final concentration) and various concentrations of "cold" Ro 15-1788 (5×10^{-10} M to 10^{-6} M final concentration) or buffer. The reactions were terminated by filtration. Binding was determined by liquid scintillation spectrophotometry, and specific binding was calculated as the difference between total and nonspecific binding. Protein content of the tissue samples was assessed using the Lowry method (13) and individual Scatchard and Hill plots were estimated by computer-aided regression analysis resulting in binding affinities (K_d) and densities (B_{max}) for the tissue from each animal.

RESULTS

Effects of Chlordiazepoxide on Punished Behavior

The initial rates of responding by the rats on the random-ratio schedule were higher than the response rates maintained by the yoked-interval schedule (Table 1). The addition of the punishment contingency decreased the response rates of five of the rats on the random-ratio schedule of food presentation resulting in similar rates and patterns of responding by both the punished and nonpunished rats (Fig. 1). For one rat (P4), however, the response rate did not change although there was an increase in the variability of his rate from session to session (Table 1). Chlordiazepoxide increased punished responding in four of the six rats (P1–P4) receiving response-contingent shock with the maximal effect occurring at a dose of 10.0 mg/kg (Fig. 2). The drug had little effect or decreased nonpunished responding by the four yoked rats. Both the punished and nonpunished responding in the other two groups showed dose-related decreases (Fig. 3).

Effects of Punishment on [^{3}H]Ro15-1788 Binding

As in the previous study described above, the addition of the punishment contingency decreased the response rates of the rats on the random-ratio schedule (Table 2).

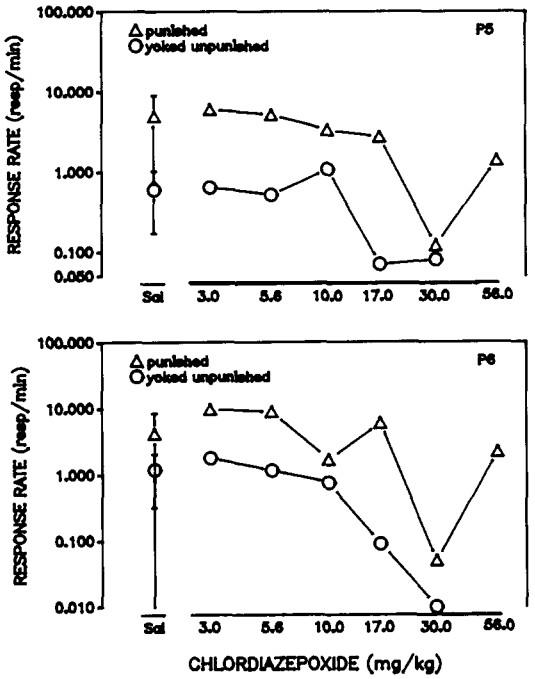

FIG. 3. Chlordiazepoxide dose-response curves for two rats for which rate increases were not observed. Other details are the same as for Fig. 2.

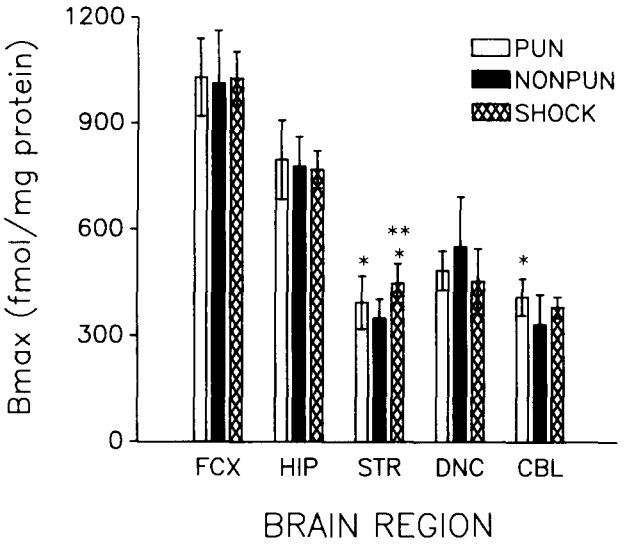

FIG. 4. Scatchard analysis of the effects of punishment on the binding of [^{3}H]Ro 15-1788 in the different brain areas for a representative animal from each of the three groups. ($p<0.05$, *compared to nonpunished, **compared to punished.)

Saturation studies carried out with [^{3}H]Ro 15-1788 showed that benzodiazepine receptor binding (B_{max}) was significantly increased in the striatum in both groups exposed to electric footshock as compared to nonshocked controls (Fig. 4). Additionally, the group given noncontingent shocks had significantly greater binding that the rats whose responding was punished. In the cerebellum, however, significant increases in binding were observed only in the group given response contingent shocks. There were no significant differences in B_{max} in any of the other brain regions examined. There were no differences in binding affinity (K_d) across groups in any of the brain regions tested (Fig. 5).

DISCUSSION

Chlordiazepoxide increased punished responding at doses that had no effect on nonpunished responding. Since the behavioral procedure used resulted in comparable response rates of both punished and nonpunished responding in the absence of drug, it is likely that this effect was punishment-specific and not rate-dependent. These results are similar to those previously reported (5, 10, 17). In the study by Jeffery and Barrett, however, nonpunished responding was also increased by chlordiazepoxide, although not to as great an extent as was punished responding. In the present study, chlordiazepoxide did not increase the response rate of two of the rats receiving response contingent footshock (P5 and P6, Table 1) possibly due to the low baseline response rates of these two rats obtained following the addition of the punishment contingency.

In the second experiment, benzodiazepine receptor binding was increased in the striatum as a result of exposure to the electric footshock and this effect was greater in the yoked group which had received noncontingent shock than in the group whose responding had been punished. These findings suggest that predictability and control of the shock may attenuate the effects of the noxious stimulus.

Indeed, many studies have shown that uncontrollable shock will have vastly different effects than will contingent shock, most notable being the behavioral depression or 'learned helplessness' which is observed following administration of a noncontingent stressor (14). Animals allowed to postpone shock also exhibit fewer stress-related effects than those who receive unavoidable shock. For example, animals given the opportunity to avoid shock

TABLE 2

RESPONSE RATES BEFORE AND AFTER THE ADDITION OF SHOCK TO THE RANDOM-RATIO RATS

	Random-Ratio		Variable-Interval		Yoked Food and Shock	
	Preshock	Shock	Preshock	Shock	Preshock	Shock
T1	62.95 ± 12.30	11.93 ± 7.68	15.33 ± 4.68	7.58 ± 2.86	0.24 ± 0.08	3.39 ± 2.71
T2	42.86 ± 8.60	8.41 ± 3.73	9.93 ± 3.17	5.55 ± 0.90	2.48 ± 2.59	3.35 ± 1.21
T3	30.41 ± 4.94	5.23 ± 1.72	12.35 ± 3.80	8.15 ± 3.19	1.04 ± 1.69	2.59 ± 1.38
T4	43.72 ± 15.29	16.04 ± 7.99	8.65 ± 3.23	5.70 ± 1.67	0.05 ± 0.05	0.14 ± 0.23
T5	52.64 ± 9.05	49.14 ± 7.87	22.92 ± 5.34	18.45 ± 3.12	0.31 ± 0.23	0.22 ± 0.23
T6	86.62 ± 9.08	25.09 ± 2.25	9.02 ± 3.95	15.04 ± 2.29	0.01 ± 0.02	0.01 ± 0.01

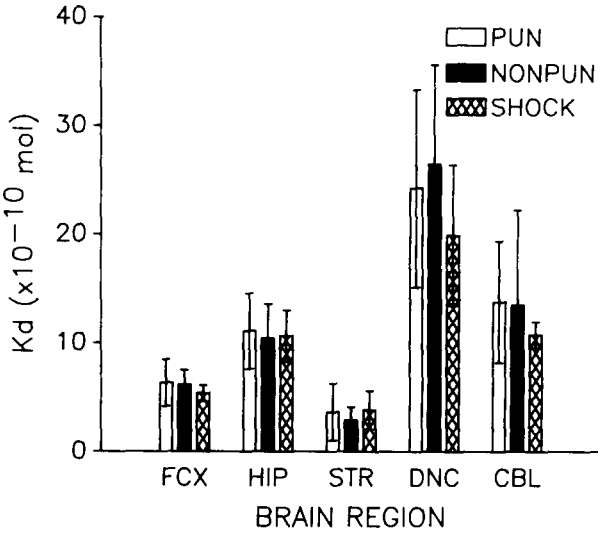

FIG. 5. Mean effect ±S.D. of punishment on the binding affinity (K_d) of [^{3}H]Ro 15-1788. Values are mean binding affinity ($\times 10^{-10}$).

exhibited fewer stomach lesions (21) and less rapid tumor growth (18) than their yoked controls who received the same shocks but could not control their delivery. In addition to these behavioral changes there is evidence of differential effects on catecholamine levels of animals receiving contingent versus noncontingent shock (22). These studies and others showing that stress decreases the density of GABA binding sites in different areas of the rat brain have suggested that the "emotional status" of the animals needs to be taken into consideration when looking at GABA or benzodiazepine receptors (1).

The second experiment also showed increases in benzodiazepine receptor binding in the cerebellum, which contains a high density of benzodiazepine receptors (23). This appears to be related to the punishing effects of electric footshock since no significant changes were seen in those rats that had received noncontingent shock. Previous reports on the effects of experimentally-induced stress on benzodiazepine receptor binding have been inconsistent. Electric footshock has been reported to decrease [^{3}H]diazepam binding in the frontal cortex (12). Conversely, increases in benzodiazepine receptor binding have been reported following immobilization stress (4) and seizures (16). Cold water swim stress has been shown in one study to increase [^{3}H]flunitrazepam binding in the cortex but not in the cerebellum (19). However, another study reported that cold water swim had no effect on benzodiazepine receptor binding in the hippocampus, occipital cortex, frontal cortex or striaum (4). Therefore, previous reports do not provide a consistent interpretation of the effects of experimentally-induced stress on benzodiazepine binding.

Thus, it appears that in addition to the ability to control the stressor, the type and duration of the stress which is used in these studies may be important factors in determining the effects of stress on benzodiazepine receptors. Additionally, it appears that different brain areas may be affected following different stress situations. In the present study, the areas which appear to be involved are the striatum and the cerebellum with no changes being observed in other brain regions such as parts of the limbic system, which contain high densities of benzodiazepine receptors and have long been thought to be involved in anxiety (23). It is possible that with finer dissections or light microscopic receptor autoradiography that such effects might be elucidated using the behavioral procedure employed.

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