Pharmacology Biochemistry & Behavior, Vol. 55, No. 3, pp. 405–413, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00110-4

Diazepam Enhances Conditioned Defeat in Hamsters (*Mesocricetus auratus*)

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Received 7 July 1995; Accepted 9 January 1996

HEBERT, M. A., M. POTEGAL, T. MOORE, A. R. EVENSON AND J. L. MEYERHOFF. *Diazepam enhances conditioned defeat in hamsters* (Mesocricetus auratus). PHARMACOL BIOCHEM BEHAV **55**(3) 405–413, 1996.—Male hamsters that have been repeatedly defeated by larger, aggressive males subsequently flee from, rather than attack, nonaggressive male intruders that are introduced into their home cages. We have referred to this generalization of flight in response to nonaggressive intruders as "conditioned defeat" (CD). In an attempt to reverse CD pharmacologically, diazepam (DZP) was administered to hamsters at two different time points relative to CD acquisition and subsequent response generalization tests, which involved the exposure of subjects to nonaggressive intruders (NAIs). In Experiment 1, subjects were given a single injection of one of 4 doses of DZP (0, 2, 6, or 20 mg/kg) immediately following CD acquisition. Twenty-four hours later, contrary to expectations, subjects that had received the 6 mg/kg dose displayed elevated flight responses in the presence of an NAI. Flight responding declined over days except in subjects that received the highest dose. In the second experiment, hamsters were administered a single injection of either 0, 2, or 6 mg/kg DZP just prior to a response generalization test occurring 24 h following CD training. Flight responses to the NAIs were again exaggerated in subjects that were given the 6 mg/kg dose, an effect that persisted several days without further drug administration. The present findings suggest the possibility that benzodiazepines can potentiate fear responses under certain stressful conditions. **Copyright © 1996 Elsevier Science Inc.**

Agonistic behavior Aggression Defeat Stress Fear Anxiety Drugs Benzodiazepines Sensitization

THE experience of defeat by a conspecific is a strong naturalistic stressor that can have profound consequences for the organism. In laboratory studies employing the rodent resident/intruder paradigm even brief defeat episodes have been shown to have strong, and in many cases lasting, effects on cardiovascular, thermoregulatory, neuroendocrine, and immunological functions (6–8,11,12,26,29). The experience of defeat also can produce dramatic changes in both nonsocial (9,15) and social behavior of rodents (24,28). In agonistic encounters, previously defeated rodents engage in species-specific defensive and submissive behaviors that serve to minimize further attack by the dominant opponent. If repeatedly defeated, especially by multiple aggressors, territorial aggression becomes completely suppressed and the animal exhibits exaggerated defensive reactions to any conspecific intruders, even nonaggressive ones. This phenomenon has been referred to as "conditioned defeat" (CD) (24,28). CD in rodents may be a useful model of human psychopathologies that involve states of extreme or inappropriate anxiety, fear, or panic (e.g., PTSD). Consistent with this idea, defeated rodents have been observed to exhibit anxiety-like behavior even in nonsocial contexts such as the elevated-plus maze (27). Understanding of the neuropharmacology of enhanced defensive reactions that result from social defeat may not only aid in the understanding of the basic neural mechanisms underlying defensive behavior but may also provide important insights into human clinical conditions.

Syrian golden hamsters are well suited for the study of defensive behavior because they display high levels of territorial aggression with minimal experimenter intervention. We have previously reported a rapid (1 day) etho-experimental technique for inducing CD in this species that involves repeated defeat trials in which naive intruder hamsters are exposed to large, aggressive residents (24). During agonistic encounters in hamsters there is an orderly sequence for the emergence of different defensive behaviors in the defeated animal (18). When a previously nondefeated hamter is faced with a dominant animal it initially exhibits side and upright defensive postures (and perhaps tails raises) when the dominant animal is in close proximity (e.g., when the dominant animal is sniffing its body). However, if such defensive behav-

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iors fail to prevent attack (and they often do if the dominant animal is highly aggressive) these defensive strategies give way to flight and escape attempts, which are a much more vigorous form of defense (18).

In contrast to hamsters without a history of defeat, hamsters that have acquired CD flee vigorously upon exposure to a novel conspecific, even if the conspecific is totally nonaggressive and the encounter occurs in the CD animal's home territory (home cage). Such "anticipatory flight" (AF), i.e., flight from a novel opponent before it attacks, is an important feature of CD in hamsters and the emergence of AF can be viewed as a fundamental shift within the animal's behavioral repertoire from defense, toward complete abandonment, of its home territory. During CD acquisition trials, we use the first display of AF as a convenient behavioral marker for the onset of CD. During response generalization tests administered on days subsequent to CD acquisition, CD-trained animals continue to flee from nonaggressive intruders despite being tested in their home cages. We have shown that flight declines over days, although the extinction rate depends on the number of additional training trials that were administered following the emergence of AF on the acquisition day (more trials yields longer extinction).

Because our procedure for inducing CD is brief, our model affords the opportunity to examine the effects of pharmacological interventions at different time points relative to CD acquisition and subsequent response generalization testing. This is an important feature because in clinical situations involving acute psychological trauma it is important, for example, to identify those anxiolytics or other psychoactive compounds that are either efficacious or counterproductive when administered immediately following the stressful event.

In the present study, we were initially interested in the post-CD acquisition administration of diazepam (DZP), the prototypical benzodiazepine (BZP) anxiolytic. We hypothesized that DZP given in this manner would reduce subsequent defensive responding because of its well established anxiolytic properties and because of its well-known amnestic effects in humans (19). DZP also has been reported to have disruptive effects on learning tasks in other animals and to interfere with long-term potentiation processes believed to underlie memory consolidation (13,22).

There have been numerous reports of BZP effects upon agonistic behavior in rodents. Most studies suggest a selective influence on defensive, rather than offensive, behavior (3), but there have been conflicting findings. For example, alprazolam, nitrazepam, and oxazepam were found to reduce the defensive behavior and escape responding of "timid" mice that were placed in the presence of a nonaggressive (group-housed) mouse (14). Similarly, chlordiazepoxide was also found to decrease defensive posturing in low-ranking hamsters in the presence of a dominant conspecific (21). On the other hand, there are examples of BZPs having generalized enhancing effects on defense as well as accounts of BZPs differentially affecting specific aspects of defensive behavior. In one study, the administration of chlordiazepoxide to mice of three strains led to increases in both defensive and submissive postures in the presence of a nonaggressive (anosmic) stimulus mouse (4). In another report, diazepam administration to intruder rats increased defensive postures but decreased submissive (supine) postures and flight (20). In other studies where BZPs were reported to decrease defensive attack of feral animals toward humans, the authors noted that flight and escape responses were either not reduced or were even elevated (3,17). It was suggested that under certain conflict conditions, BZPs may lead to a shift within the repertoire of defensive behaviors from defensive attack to flight and escape (3).

In most of the experiments just mentioned, the effects of BZPs have been evaluated while subjects were under the influence of the drugs. We, however, administered DZP immediately following the defeat experience. This strategy mimics human situations in which an anxiolytic drug such as a BZP might be given immediately following the experience of a psychologically traumatic event. Because subjects were then tested 24 h later without further drug administration, we could evaluate the efficacy of the drug treatment without the potential problem of drug effects on motor performance. In the first experiment we gave a single injection (of one of several doses) of DZP immediately following CD acquisition then examined the responses of the subjects to the presentation of a nonaggressive intruder on subsequent days without further drug administration. Because we were surprised to observe exaggerated flight responses in this experiment, a second was completed in which CD subjects were given response generalization tests under the acute influence of the drug. We report that DZP had the effect of exacerbating CD in both experiments. These findings raise the possibility that, in certain species or in certain types of stressful situations, BZPs might enhance or sensitize fear-like responding.

EXPERIMENT 1

Introduction

In the present experiment, hamsters were given a single injection of one of several doses of DZP immediately following the last CD training trial. A high dose (20 mg/kg) was included in an attempt to maximize the likelihood of memory disruption. The first generalization test occurred 24 h later, so even this high a dose would not have directly affected motor ability at the time of testing. Another generalization test was given 5 days following CD training, without further drug administration.

Method

Animals and Husbandry. All animals were male Syrian golden hamsters (*Mesocricetus auratus*) obtained from Charles River Co. Forty-eight experimentally naive hamsters served as subjects. They were 50–60 g at the time of arrival and 110–130 g at the beginning of the experiment. They were first maintained in triplets, then singly housed at least 1 week prior to CD training. A group of 18 hamsters, 50–60 g at the time of arrival and 100–130 g at the start of the experiment, served as nonaggressive intruders. They were always housed in triplets to minimize aggressiveness. Ten larger males (130–180 g) were used as aggressors (see below). Additionally, pretrained CD hamsters that were not subjects of present study were used to "prime" attack in the aggressors prior to CD acquisition trials (described below). All animals were maintained under a reverse 14 L:10 D cycle, lights off at 0900 h, and given free access to food and water at all times except during testing.

Procedure

Selection of Aggressors. To minimize the number of trials needed to induce CD in the subjects and to reduce variability in the number of trials across subjects, we were careful to select (prior to the experiments) only highly and consistently aggressive animals from a colony of approximately 40 isolated males. This was accomplished by ranking the aggressors ac-

cording to the number of attacks exhibited against standardized male intruders treated with the sedative/analgesic methotrimeprazine (Levoprome, Lederle, 2 mg/kg, IM) (23). The 10 animals that most consistently attacked these intruders over several screening trials were used as aggressors in the current experiment. To further increase the probability that aggressors would attack subjects during CD acquisition trials, the aggressors were "attack-primed" (25) prior to their use in the CD acquisition trials by briefly exposing them to pretrained, fleeing CD intruders (10). The CD animals used for attack priming purposes were not subjects of the present study.

Preparation of nonaggressive intruders (NAIs): In addition to being group housed, a procedure that reduces aggression in hamsters, animals that were used as NAIs were treated with a high dose of diazepam (15 mg/kg, IP) 30 min before use in the experiments. We have reported elsewhere that these animals provide a very workable solution to controlling the problem of variability in the behavior of stimulus animals, which can alter the subject's behavior (24). At this dose, DZP-treated intruders exhibit stereotypy in the form of pacing back and forth, usually along one wall of the cage at a time. They pay little or no attention to other animals, yet they are adequate stimuli for eliciting defensive or aggressive reactions in other animals (e.g., CD hamsters flee vigorously from them) (24).

Experimental Design. Half of the subjects ($n = 24$) were randomly assigned to the CD condition, the remaining to the handled control condition. Within each of these conditions, subjects were randomly assigned ($n = 6$) to one of four groups given either 0 (vehicle), 2, 6, or 20 mg/kg DZP.

CD Acquisition. All trials were conducted during the first 3 h of darkness when activity and aggression levels in hamsters are highest (16). Each subject of the CD groups received a series of 5 min defeat trials, separated by 6-min intervals. During each trial, a subject was placed into the home cage of an aggressor for 5 min. Just prior to each trial, except the first, subjects were exposed to an NAI for 1 min to determine whether the subject displayed "anticipatory flight" (AF), i.e., flight not precipitated by an attack. Once the subjects exhibited AF, they were given one additional defeat trial with an aggressor and returned to their home cages. To control for effects of handling, non-CD control subjects were transported from their home cages to an empty neutral cage (and back) the same number of times the CD subjects received defeat trials.

Drug Administration and Generalization Tests. Immediately following the last defeat trial, or transport for controls, subjects were given a single IP injection of either vehicle, 2, 6, or 20 mg/kg diazepam (1 ml/kg) and returned to their home cages. [Diazepam (Sigma) was suspended by ultrasonication in a vehicle consisting of 2 drops of Tween 80/10 ml distilled water.] The next day (24 h later) and 5 days following CD training, each subject was given a response generalization test (without further drug administration). During these 5-min tests an NAI was placed into the home cage of the subject and an observer blind to the subject's condition recorded the frequency of occurance of the following defensive behaviors commonly exhibited by this species (11): 1) flight: rapid movements away from opponent, either horizontal or vertical, including escape attempts; 2) defensive postures: upright and side defense posture in proximity of the intruder; 3) tail raises: tail lifted with or without hind limb adduction.

The number of approaches subjects made toward the NAI was also recorded. In addition, species-typical offensive behaviors were scored: upright or side attack, biting, aggressive grooming (mouthing of opponent's fur), and flank marking (11).

Results

An alpha level of 0.05 was adopted for all statistical tests that were performed using commercially available software (1).

CD Acquisition. The mean number of conditioning trials administered to subjects was 2.7 (SE = 0.21). None of the CD groups received significantly more trials than any other, $F(3, 20) = 1.32$. The number of CD acquisition trials received by each of the groups is given in Table 1 along with the frequency of flight and defensive responses per trial. Also listed are the mean number of attacks and bites received by the subjects per trial. F-tests revealed no significant differences among the four CD groups on any of these measures ($0.96 < Fs < 2.53$; $0.09 < ps < 0.43$).

Generalization Tests. For each behavior, a 2 (CD) × 4 (dose) × 2 (test) mixed ANOVA, test being the only repeated factor, was performed on the frequency data. Specific group comparisons were made using Tukey HSD tests.

Flight. Figure 1 illustrates the mean flight responses of subjects in each group during the generalization tests.

Flight levels were significantly higher in CD than in non-CD groups across both test days, $F(1, 40) = 29.67$, $p < 0.001$. There was a significant interaction among the three variables, $F(3, 40) = 4.63$, $p < 0.01$. During the first generalization test, there were significantly more flights in the 6 mg/kg CD group than in each of the other CD groups; the latter groups did not differ from each other but were higher than non-CD controls. By the second test, flight declined significantly in the 6 mg/kg CD group to levels of the other CD groups, which still exceeded non-CD controls at all drug doses. On the second test, the 20 mg/kg CD group tended toward higher flight levels than the other CD groups, but the differences did not reach statistical significance. Of the few observations of flight in non-CD animals, all occurred in animals given either of the two highest doses. One non-CD subject given 20 mg/kg DZP displayed very high flight responses on both generalization tests, which accounted for the relatively high means of this group.

Defensive Postures. The mean number of upright and side defensive postures were combined within each group (see Table 2). There was a significant main effect of CD, $F(1, 40) = 6.18$, $p < 0.01$, and test, $F(1, 40) = 15.03$, $p < 0.001$, upon defensive responding, as well as a significant CD × test interaction, $F(1, 40) = 17.76$, $p < 0.001$. During the first test, there were no significant differences among the CD and non-CD groups; all had low frequencies of defensive responses. In the second test, the collective responses of the CD groups increased significantly compared to both test 1/CD and test 2/non-CD values. However, the 6 mg/kg CD group was the only group to exhibit a significant increase over test days.

Tail Raises. There was a main effect of CD upon the frequency of tail raises, $F(1,40) = 36.60$, $p < 0.01$, but no main effect of test, $F(1, 40) = 0.15$ (see Table 2). On both test days there were significantly more instances of tail raises in the CD than in the non-CD groups. The ANOVA indicated a nearly significant main effect of dose, $F(3, 40) = 2.40$, $p = 0.08$ and a significant CD × dose interaction, $F(3, 40) = 3.18$, $p < 0.05$. The main effect of dose resulted from a higher overall number of tail raises in the vehicle group than in the 2 mg/kg group. However, this difference was evident in CD but not in non-CD controls; hence, the CD × dose interaction. When test days were examined, there were no significant differences among the CD groups on either day of testing according to the Tukey HSD test. Non-CD controls were also unaffected by dose on both test days.

Approaches. Overall, CD animals approached intruders

TABLE 1

EXPERIMENT 1: MEAN (SE) NUMBER OF CD ACQUISITION TRIALS IN EACH DRUG GROUP AND BEHAVIORAL RESPONSES OF SUBJECTS AND AGGRESSORS DURING CD ACQUISITION TRIALS (BEFORE DRUG ADMINISTRATION)

		Subjects			Aggressors	
CD Group	Number Trials	Flight	Defensive Postures	Tail Raises	Attack	Bite
Vehicle	2.3 (0.2)	19.5 (2.5)	7.2 (1.3)	9.5 (0.6)	11.4 (1.1)	8.2 (1.0)
2 mg/kg	2.8 (0.5)	24.9 (3.8)	9.7 (1.2)	11.1 (1.0)	14.1 (1.6)	9.1 (1.6)
6 mg/kg	3.3 (0.6)	19.8 (2.8)	6.9 (1.4)	10.1 (0.7)	12.1 (0.9)	9.1 (1.5)
20 mg/kg	2.3 (0.2)	13.9 (2.2)	4.9 (1.0)	9.0 (0.7)	10.4 (1.4)	6.4 (0.9)

significantly less often than non-CD controls, $F(1, 40) = 17.44$, $p < 0.001$ (see Table 2). There were also significantly more approaches on the second test day than the first, $F(1, 40) = 5.42$, $p < 0.05$. However, the CD × days interaction was significant, $F(1, 40) = 11.75$, $p < 0.001$. That is, there were significantly fewer approaches in the CD groups than in the non-CD controls on the first day but not the second. There was no main effect of dose upon the number of approaches toward intruders, $F(3, 40) = 0.5$, nor was there a significant CD × dose interaction, $F(3, 40) - 2.15$. However, on day 1 CD animals given either 6 or 20 mg/kg DZP exhibited significantly fewer approaches than similarly treated non-CD groups. By the second day, levels increased in CD animals to those of non-CD controls.

Offensive Behavior. There were few occurrences of any particular offensive behavior. The numbers of attacks, bites, aggressive grooms, and flank marks were, therefore, combined for each animal to give composite scores that were then subjected to a three-way ANOVA. Overall, CD subjects displayed virtually no offensive responses; their scores were significantly lower than scores of non-CD animals, $F(1, 40) = 10.85$, $p < 0.01$ (see Table 2). There was no main effect of dose, $F(3, 40) = 1.68$, nor any significant interactions involving dose. However, there were significantly more offensive behaviors observed on the second test day than on the first, $F(1, 40) = 4.59$, $p < 0.05$, which was due mostly to a significant rise in offensive responding in the vehicle nonCD group. During test 2, this group was significantly higher than all other groups except the 2 mg/kg non-CD group. Offensive responses remained low over tests in the CD groups and in non-CD animals given DZP, especially the higher doses; on test 2, there were significantly fewer responses in the non-CD groups given 6 or 20 mg/kg DZP than in the non-CD group given vehicle.

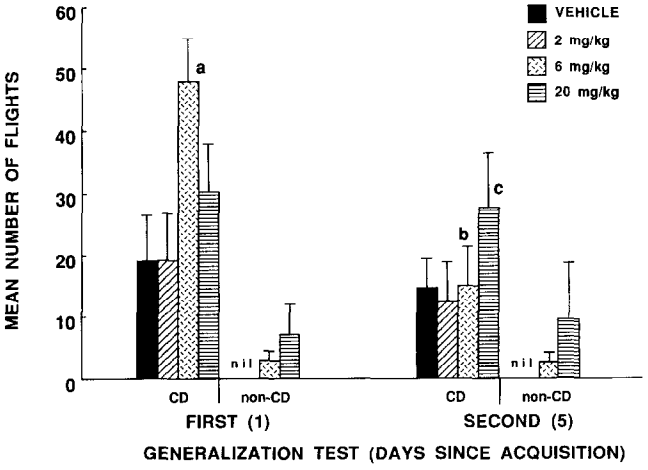

FIG. 1. Mean number of flights (with SEs) for each group during generalization tests in Experiment 1. Each animal received a single drug injection that was administered immediately following CD training, 24 h prior to first generalization test. [a]Significantly greater than every other group on first test day. [b]Significant decrease from levels of this group on first test day. [c]Only CD group on second test day to exceed corresponding non-CD group. All CD groups exceeded respective non-CD groups on first test day and there were no differences among non-CD groups on either test day. $n = 6$ for all groups. nil = groups in which no flight was observed on a given day.

Discussion

We were surprised to discover that DZP administration immediately following CD acquisition did not reduce subsequent flight responding but, instead, exacerbated it on subsequent test days, without additional drug treatment. During the first generalization test, 1 day after CD acquisition and drug administration, CD animals that had received 6 mg/kg DZP displayed flight levels two to three times greater than those of CD animals that were given either vehicle or the low dose (2 mg/kg) of DZP. The CD animals given the highest dose (20 mg/kg) of DZP had the second highest levels of flight on the first generalization test, suggesting an inverted-U shaped dose–response function with this behavior. By the fifth day postacquisition flight levels declined in CD animals that had been given 6 mg/kg DZP (5 days prior). However, the CD animals given the 20 mg/kg dose retained high levels of flight indicating persistence over days in the effects of DZP upon fear responding.

There was an inverse relationship between the occurance of flight and defensive postures, with groups having the most flight exhibiting the least defensive postures. There is a clear hierarchy of defensive responses in Syrian golden hamsters and a very orderly sequence for the emergence of different defensive behaviors (18). Prior to attack by a dominant animal, previously nondefeated hamsters will typically exhibit upright and side defensive postures when the dominant animal is in close proximity (e.g., when the dominant animal is sniffing its body). However, once the dominant animals attacks or bites the now subordinate, defensive postures give way to overt flight and/or escape attempts (18). The replacement of defensive postures by flight is, therefore, an indicator of high defensiveness (fearfulness). CD animals fail to exhibit the normal sequence of defensive behavior upon exposure to a novel conspecific. Instead of exhibiting defensive postures when first presented with a novel NAI, they instead display flight even though they are never attacked. Therefore, our observation of a reduction in defensive postures coupled by an increase

TABLE 2

EXPERIMENT 1: MEAN (SE) FREQUENCIES OF DEFENSIVE POSTURES, TAIL RAISES, APPROACHES TO NAI, AND OFFENSIVE BEHAVIORS OF SUBJECTS DURING GENERALIZATION (GEN) TESTS

		Defensive Postures		Tail Raises		Approaches to NAI		Offensive Behaviors	
Gen Test	Dose	CD	non-CD	CD	non-CD	CD	non-CD	CD	non-CD
ONE									
	0.00	5.2 (2.2)	3.0 (1.1)	13.2* (1.8)	0.0 (0.0)	7.5 (1.8)	12.2 (0.5)	0.0 (0.0)	1.3 (1.0)
	2.00	6.0 (2.2)	4.0 (0.9)	8.5 (1.8)	1.0 (0.4)	6.2 (1.4)	10.3 (0.6)	0.0 (0.0)	2.2 (1.0)
	6.00	1.8 (0.8)	6.7 (1.8)	14.2* (2.1)	3.3 (1.3)	3.7* (1.5)	13.3 (2.1)	0.0 (0.0)	0.2 (0.2)
	20.00	2.2 (1.2)	5.0 (1.9)	7.7 (2.5)	4.7 (2.6)	5.8* (2.0)	16.0 (2.8)	0.0 (0.0)	0.3 (0.2)
TWO									
	0.00	12.7* (3.2)	3.3 (1.1)	16.0* (2.3)	0.2 (0.2)	9.5 (1.6)	12.2 (2.0)	0.0* (0.0)	4.3 (2.1)
	2.00	11.8* (2.9)	3.2 (1.4)	8.5 (3.0)	0.5 (0.3)	13.3 (2.6)	9.8 (1.1)	0.0 (0.0)	2.3 (1.0)
	6.00	11.3‡ (3.0)	5.2 (1.6)	11.2 (1.7)	3.2 (1.9)	9.8 (2.6)	12.3 (1.4)	0.2 (0.2)	0.5† (0.3)
	20.00	7.3 (2.7)	5.8 (1.6)	13.3* (2.2)	2.7 (1.9)	9.7 (2.0)	13.8 (2.1)	0.8 (0.7)	0.8† (0.6)

DZP administered immediately following CD training, 24 h prior to first gen test. $n = 6$ for all groups.
*Group significantly different from corresponding nonCD group on same day.
†Group significantly different from 0.00 mg/kg (vehicle) group on same day.
‡Group significantly different from its day one level.

in flight clearly suggests enhanced rather than diminished defensiveness, and arguably fearfulness, in the CD subjects given DZP.

CD animals given 6 or 20 mg/kg DZP also made fewer approaches toward the intruder, particularly during the first generalization test. Moreover, DZP suppressed offensive responding in non-CD subjects although, for unknown reasons, there did not appear to be an effect of drug dose on the frequency of tail raises. On balance, the pattern of results suggest that DZP, particularly at the higher doses, exacerbated conditioned fear responding in hamsters when administered immediately following CD acquisition.

These behavioral effects were observed 24 h following administration of DZP. Because little is known about DZP pharmacokinetics in hamsters, we can only speculate about the mechanisms that might be involved. BZPs are rapidly absorbed but quickly redistributed out of the brain (2). If one assumes that the drug had been eliminated by the time of the first generalization test, one possibility is that changes (downregulation) in benzodiazepine or GABA receptors may have accounted for the observed effects.

EXPERIMENT 2

Introduction

Given these surprising results, we wanted to examine the responses of CD subjects under the acute influence of DZP. As noted earlier, there have been mixed reports of acute BZP effects upon defensive and submissive behavior in rodent species such as mice and rats. To our knowledge, there has been only one report of BZP effects upon agonistic behavior in hamsters (21). In that experiment, a high dose (50 mg/kg) of chlordiazepoxide was found to reduce defensive and submissive behavior in subordinate hamsters paired with non-drugged dominant counterparts. In the present experiment, we administered DZP just prior to the first generalization test, which occurred 24 h following CD training. We did not administer the highest (20 mg/kg) dose of DZP to subjects in this experiment because we did not want to induce stereotypy in subjects undergoing social testing.

Method

Animals and Husbandry. Thirty and 18 hamsters were used as subjects and NAIs, respectively. The subjects of this experiment were the same species, sex, and weight as subjects in Experiment 1; they were also from the same supplier and were housed under the same conditions.

Procedure

Experimental Design. Two treatment conditions, CD and non-CD (handled) controls, were completely crossed with three drug treatments: vehicle, 2 mg/kg, and 6 mg/kg. An equal number of subjects ($n = 5$) was randomly assigned to each of the six independent groups.

CD Acquisition, Drug Administration, and Generalization Tests. Each subject in the CD condition underwent the CD acquisition regimen described in the first experiment. Non-CD controls were handled as in Experiment 1.

Both CD and non-CD subjects were given three generalization tests in which an NAI was placed in the subject's home cage for 5 min. These took place 1, 2, and 5 days following CD acquisition (or following handling in the case of the controls). Subjects were given a single IP injection of either vehicle, 2 mg/kg, or 6 mg/kg DZP (1 ml/kg) 30 min prior to the first, and only the first, generalization test. During each test a trained observer, blind to the subject's condition, recorded the subject's responses as in Experiment 1.

Results

Statistical tests were again considered significant at the conventional $p < 0.05$ level. The same statistical software was used for data analysis (1).

CD Acquisition. The mean number of acquisition trials administered to subjects was 2.7 (SE = 0.32). There were no significant differences in trials to criterion among the three CD drug groups, $F(2, 12) = 2.33$. Table 3 gives the mean number of trials received by each group as well as the frequency of flights and defensive postures per trial exhibited by subjects during acquisition. Table 3 also shows the mean number of attacks and bites per trial received by the subjects

TABLE 3

EXPERIMENT 2: MEAN (SE) NUMBER OF CD ACQUISITION TRIALS IN EACH DRUG GROUP AND BEHAVIORAL RESPONSES OF SUBJECTS AND AGGRESSORS DURING CD ACQUISITION TRIALS (BEFORE DRUG ADMINISTRATION)

		Subjects			Aggressors	
CD Group	Number Trials	Flight	Defensive Postures	Tail Raises	Attack	Bite
Vehicle	3.2 (0.6)	24.3 (4.3)	7.0 (1.7)	15.8 (1.8)	11.0 (1.6)	5.0 (0.6)
2 mg/kg	2.8 (0.4)	29.5 (4.5)	7.0 (1.2)	16.6 (2.4)	13.3 (1.8)	5.5 (1.3)
6 mg/kg	2.0 (0.0)	30.5 (5.3)	7.8 (1.8)	16.8 (1.2)	13.1 (2.1)	5.3 (0.9)

during training. One-way ANOVAs showed that the three CD drug groups did not differ significantly on any of these behavioral measures ($0.10 > Fs > 0.50$, $0.62 > ps > 0.94$).

Generalization Tests. The data for each category of behavior were treated similarly. A three-way mixed ANOVA, with CD and dose as between-subjects factors and test as the within-subjects factor, was performed using the frequency data of each behavior (or combination of behaviors) listed below. Tukey HSD tests were used to make specific comparisons among the experimental groups.

Flight. The flight responses of subjects across the three generalization tests are shown in Fig. 2. CD subjects exhibited significantly more flights than non-CD subjects, $F(1, 24) = 33.70$, $p < 0.001$. Flight levels declined significantly over days, $F(2, 48) = 8.53$, $p < 0.001$. There was a main effect of drug dose upon flight levels, $F(2, 24) = 12.28$, $p < 0.001$, and a significant CD × dose interaction, $F(2, 24) = 8.78$, $p < 0.001$. In CD animals, DZP administration led to a dose-related enhancement of flight. Across all three test days, the 6 mg/kg CD subjects had the highest flight levels. On the first two tests, this group exhibited significantly more responses than the vehicle CD group. On only the second test were the levels significantly greater than the 2 mg/kg CD group. By the third test, levels in the 6 mg/kg CD group dropped significantly compared to the first test. However, flight responses on test 3 still remained higher in this group than in the other two CD groups, although the differences were not statistically significant. Vehicle and 2 mg/kg CD groups exceeded non-CD controls only on the first test. Although non-CD animals given vehicle never exhibited flight, it was occasionally observed in non-CD animals given DZP, particularly the 6 mg/kg dose.

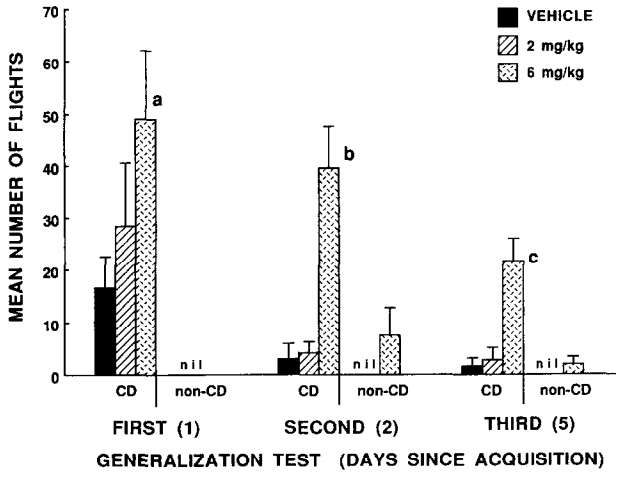

FIG. 2. Mean number (with SEs) of flights for each group during generalization tests in Experiment 2. Each animal received a single drug injection that was administered 24 h following CD training, 30 min prior to the first generalization test. [a]Significantly greater than all other groups on day one except 2 mg/kg CD group. [b]Significantly greater than all other groups on second test day. [c]Significant decrease from levels on first test day. $n = 5$ for all groups. nil = groups in which no flight was observed on a given day.

Defensive Postures. The frequencies of upright and side defensive postures were combined for each group and are presented in Table 4. According to the three-way ANOVA, CD animals displayed significantly more defensive postures overall than non-CD controls, $F(1, 24) = 15.55$, $p < -.001$. Levels of defense declined significantly over test days, $F(2, 48) = 11.39$, $p < 0.001$. There was no main effect of dose, $F(2, 24) = 1.75$, although the CD × dose, $F(2, 24) = 3.11$, $p = 0.061$, and dose × test, $F(4, 48) = 2.47$, $p = 0.057$, interactions were marginally significant. During the first test, there was a trend for a dose-dependant reduction in defensive responding of CD animals: defensive behavior in the 6 mg/kg CD group was less than that in the 2 mg/kg CD group, which was less than that in the vehicle CD group. Statistically, however, only the 6 mg/kg CD group and the vehicle CD group differed. Also during the first test, the vehicle and 2 mg/kg CD groups, but not the 6 mg/kg CD group, had significantly more defensive responses than non-CD controls. By test 2, levels in the vehicle and 2 mg/kg CD groups dropped significantly to those of the 6 mg/kg CD group, which remained as low as the non-CD control groups. Levels remained equally low during the third test in all groups.

Tail Raises. There was a significant main effect of CD, $F(1, 24) = 42.13$, $p < 0.001$, dose, $F(2, 24) = 4.25$, $p < 0.05$, and test, $F(2, 48) = 3.13$, $p < 0.05$ on tail raise frequency (see Table 4). The CD × test, $F(2, 48) = 10.08$, $p < 0.001$, and dose × test, $F(4, 48) = 5.93$, $p < 0.001$, interactions were also significant as was the three-way interaction, $F(4, 48) = 2.60$, $p < 0.05$. During the first test, the CD groups had significantly more tail raises at each dose than their respective drug-treated controls. There were no differences among the CD groups nor within the non-CD groups. By test 2, the frequency of tail raises declined significantly to those of controls in the CD subjects given vehicle and 2 mg/kg DZP, but not in CD subjects given 6 mg/kg DZP. During the third test, frequencies in the 6 mg/kg CD group remained elevated above the other CD groups and controls, which were equally low. Tail raises were virtually nonexistent in the non-CD vehicle group but were displayed by some non-CD subjects given DZP (especially 6 mg/kg).

Approaches. Table 4 summarizes the frequency of approaches toward the intruder in each group. There was no main effect of either CD, $F(1, 24) = 2.75$, or dose, $F(2, 24) =$

TABLE 4

EXPERIMENT 2: MEAN (SE) FREQUENCIES OF DEFENSIVE POSTURES, TAIL RAISES, APPROACHES TO NAI, AND OFFENSIVE BEHAVIORS OF SUBJECTS DURING GENERALIZATION (GEN) TESTS

Gen Test	Dose (mg/kg)	Defensive Postures		Tail Raises		Approaches to NAI		Offensive Behaviors	
		CD	non-CD	CD	non-CD	CD	non-CD	CD	non-CD
ONE									
	0.00	14.8* (4.0)	2.0 (2.0)	13.2* (2.9)	0.0 (0.0)	14.2 (2.8)	14.4 (2.8)	0.4 (0.4)	6.8 (3.5)
	2.0	10.0* (1.1)	0.8 (0.6)	15.6* (3.5)	0.0 (0.0)	17.8 (1.6)	15.2 (1.5)	0.0 (0.0)	7.8 (3.6)
	6.0	5.8† (1.2)	1.2 (0.5)	12.0* (2.9)	0.0 (0.0)	12.0 (3.0)	17.2 (2.8)	0.0 (0.0)	7.6 (2.8)
TWO									
	0.0	4.4‡ (1.0)	1.8 (0.8)	4.0‡ (2.9)	0.0 (0.0)	16.8 (3.9)	9.8 (1.8)	1.0 (0.5)	8.8 (2.4)
	2.0	2.4‡ (0.5)	2.0 (0.6)	5.8‡ (3.0)	1.2 (1.2)	10.2 (0.9)	15.8 (2.4)	0.2 (0.2)	4.2 (1.6)
	6.0	0.2 (0.2)	2.4 (1.2)	13.8*‡ (1.6)	5.2 (3.2)	2.6*‡ (0.7)	13.8 (2.0)	0.0 (0.0)	2.6 (1.8)
THREE									
	0.0	4.2‡ (1.6)	2.0 (0.8)	4.0‡ (2.4)	0.0 (0.0)	13.0 (3.3)	12.4 (2.1)	17.4‡ (10.1)	8.4 (3.3)
	2.0	3.8‡ (2.6)	1.0 (0.6)	5.2† (2.4)	0.0 (0.0)	11.8 (3.4)	12.4 (2.2)	10.6 (5.3)	10.2 (1.8)
	6.0	3.6 (1.9)	4.4 (2.4)	16.6*‡ (2.2)	2.2 (2.0)	6.0 (1.9)	12.2 (2.0)	0.0‡ (0.0)	9.4 (3.8)

DZP administered 30 min prior to first generalization test, 24 hours following CD training. $n = 5$ per group.
*Group significantly different from corresponding non-CD group on same day.
†Group significantly different from 0.00 mg/kg (vehicle) group on same day.
‡Group significantly different from its day 1 levels.

2.59, upon this measure. The CD × dose interaction reached statistical significance, $F(2, 24) = 5.37, p < 0.01$. In CD but not in non-CD subjects, DZP led to a dose-dependent reduction in the number of approaches. On the second test day, CD subjects given 6 mg/kg had significantly fewer approaches than vehicle, but not 2 mg/kg, CD subjects. The 2 mg/kg CD group had fewer approaches than the vehicle CD group, but the difference was not significant.

Offensive Behavior. There were few occurrences of any given offensive behavior so the numbers of attacks, bites, offensive grooms, and flank marks were again combined. More non-CD than CD animals exhibited these behaviors, particularly during the first two tests (see Table 4). By the third test nearly half of the CD subjects in the vehicle and 2 mg/kg groups (4 of 10) showed some offensive behavior, whereas none (0 of 5) of the 6 mg/kg subjects ever exhibited any of the offensive behaviors. Overall, the frequency of offensive behavior was significantly lower in CD groups than in non-CD groups, $F(1, 24) = 4.69, p < 0.05$. There was also a main effect of tests, $F(2, 48) = 8.08, p < 0.001$. Scores on test 3 were significantly higher than those on tests 1 and 2, which did not differ. There was not a significant main effect of dose, $F(2, 24) = 0.55$, nor were any of the interactions statistically significant. However, the score of zero in the 6 mg/kg CD group on the third test proved to be significantly lower than that of the vehicle CD group on that day.

Discussion

It is clear from the present results that DZP did not attenuate CD. Instead, DZP, particularly the 6 mg/kg dose, increased the intensity of fear responses in CD animals. Under acute influence of the drug, CD subjects given 6 mg/kg DZP displayed nearly three times the number of flights exhibited by CD animals given vehicle. An intermediate amount of flight was observed in CD animals given the lower (2 mg/kg) dose.

As in Experiment 1, animals that displayed the most flight tended to exhibit fewer defensive postures. Again, this reflects changes in flight distance in the highly fearful (fleeing) animals; they fled at distances in which less fearful animals would have exhibited defensive postures (18).

Even though DZP was administered only once, 6 mg/kg CD subjects continued to display signs of enhanced fear over subsequent generalization tests. One day following drug administration flight levels were still high in this group but declined by the last test day. However, there was evidence of residual fear responding on the last generalization test. Despite lower flight levels during test 3, tail raises remained high in the 6 mg/kg CD group on all three test days. These subjects also made the fewest approaches toward the intruder across all generalization tests. Also, whereas there was a trend for territorial aggression to emerge by the last generalization test in the other CD groups, the 6 mg/kg CD animals never exhibited any evidence of offensive behavior.

GENERAL DISCUSSION

The present findings appear robust, having been demonstrated in two different experiments. The results are, however, difficult to reconcile with the voluminous reports of anxiolytic-like effects of BZPs in numerous animal models of anxiety (30). For example, BZPs have been found to increase punished operant responding in conflict models (30). However, there have been important, if often neglected, criticisms leveled at many of these procedures (30). In conflict models, for example, it is difficult to distinguish drugs that reduce anxiety from ones that enhance motivation or drive for the positive reinforcers employed in the procedures, as either can produce the same set of results. As it turns out, BZPs have been shown to have potent appetite enhancing effects (32).

Nevertheless, BZPs have produced antianxiety effects in other animal models that do not involve appetitive responding. For example, DZP was shown to prevent the natural tendency of rats to bury objects associated with the delivery of aversive stimuli, so-called "defensive burying" (31). However, even in the defensive burying model there are apparently limitations on the effectiveness of BZPs, which may be especially relevant to the present study. The author noted that the suppressive

effects of DZP upon defensive burying were completely eliminated when higher shock intensities were employed (30). What this suggests is that BZPs may be ineffective under extremely aversive conditions.

Hamsters in our CD model are exposed to repeated, high-intensity conflict in a very short time period. We have speculated that emotional responding by CD hamsters may be similar in some respects to exaggerated defensive reactions observed in humans exposed to severe, traumatic events (e.g., PTSD patients). To the extent that such comparisons are valid, our findings may have significant clinical implications. These observations, along with the present experimental data, raise the question of whether the use of BZPs in some cases of severe, emotional trauma may actually sensitize reactions to subsequent stressors. Manipulation of the benzodiazepine/GABA receptor complex might lead to changes in baseline levels of anxiety. Humans diagnosed with panic disorder (with agoraphobia) scored even higher on anxiety questionnaires following the discontinuation of BZP therapy (5).

An alternative interpretation of the present findings might be that Syrian golden hamsters are anomalous in their responses to benzodiazepines, although there has been at least one earlier report of chlordiazepoxide having attenuating effects on defensiveness in this species (21). A possible important difference between that study and ours, aside from the type of benzodiazepine used, is that we assigned subjects to defeat conditions a priori. This strategy eliminates "self-selection" bias that occurs when animals are simply paired and the combatants are the principle determinants of the outcome (winner and loser). We were also careful to ensure behavioral homogeneity among the CD subjects before drug manipulations by employing a specific behavioral endpoint, AF, during CD acquisition trials. The exposure of subjects to multiple aggressors in series was also unique to the present experiment and may have contributed substantially to the severity of the defeat experience. Higher levels of fearfulness were probably induced by our procedures.

There have been a few reports of apparent fear-enhancing effects of BZPs in other species under certain circumstances. Although contradictory data have been reported (20), there have been accounts of increased defensive and submissive responses with BZP administration in some strains of mice (4). Also, enhanced flight from approaching humans has been observed in feral animals (rats and cats) treated with BZPs (3,17). In one of these reports, the authors' explanation for their findings was that BZPs produce shifts within the animal's repertoire of defensive responses away from defensive attack toward defensive flight (3). Such a shift in behavior may have resulted from an elevation in fear motivation with BZP treatment. Taken with the present findings, such observations suggest that the administration of BZPs to highly defensive animals can further increase levels of underlying fear motivation.

However, there were hints within the present data that suggested that DZP may have fear-sensitizing effects even in nonstressed hamsters. In both experiments, there was a tendency for non-CD animals to exhibit signs of fear if they had received DZP, especially at the higher doses. Of the non-CD animals, only DZP treated subjects displayed any flight. They also exhibited consistently, albeit not significantly, more defensive and submissive postures than nondrugged controls, while their offensive behavior tended to be lower. It appeared that non-CD hamsters administered DZP were poised to exhibit fear responses. In hamsters with CD, therefore, existing high levels of fear from the defeat encounters may have acted synergistically with fear-sensitizing effects of DZP in this species to produce the extreme flight responses that were observed.

At this time we can only speculate about the neural mechanisms underlying our findings. It would, of course, be particularly informative to examine benzodiazepine receptor inverse-agonists (beta carbolines) and antagonists (flumazenil), either alone or in combination with DZP, in our hamster CD model. Also, studies are needed to determine the species generality of the present findings. We have recently developed a similar CD model using C57BL/6 and DBA/2 mice to further evaluate the DZP effect. The potential clinical significance of these findings certainly warrants further investigation.

ACKNOWLEDGEMENTS

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH Publication 85-23, revised 1985. All procedures were reviewed and approved by the WRAIR Laboratory Animal Care and Use Committee. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (Para 4-3, AR 360-5).

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