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Pharmacology, Biochemistry and Behavior 67 (2000) 433–447

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

Effects of social defeat and of diazepam on behavior in a resident–intruder test in male DBA/2 mice☆

Lucille A. Lumley*, Raquel F. Charles, Richelle C. Charles, Mark A. Hebert, Denise M. Morton, James L. Meyerhoff

Department of Neurochemistry and Neuroendocrinology, Division of Neuroscience, Walter Reed Army Institute of Research, Building 503, Robert Grant Avenue, Walter Reed Army Medical Center, Washington, DC 20307-5100, USA

Received 7 March 2000; received in revised form 20 June 2000; accepted 6 July 2000

Abstract

Social stress induces robust behavioral and physiological changes, some of which may alter the responsiveness to pharmacological agents, including diazepam (DZP). We used a resident–intruder paradigm to (1) develop a comprehensive ethogram of behavioral changes following social defeat (SD) in the socially reactive strain, DBA/2 male mice, (2) determine whether acute exposure of DBA/2 mice to low-dose DZP would induce flight or aggressive behavior, both of which have been observed in other rodent models and (3) to test whether prior social stress affects responses to DZP. Behavioral responses to a nonaggressive intruder (NAI) mouse 24 h post-SD were measured in resident subject mice exposed to DZP (0, 0.5, 2.0 mg/kg, ip) either prior to the resident–intruder test (Experiment 1) or immediately post-SD (Experiment 2); control mice were not defeated (NOSD). In general, SD mice displayed increased passive and active avoidance, defense, immobility, and risk assessment relative to NOSD mice. In Experiment 1, mice treated acutely with 0.5 mg/kg DZP had more approach and flight behavior, while those treated with 2.0 mg/kg DZP had more avoidance than vehicle-treated mice, independent of SD. In Experiment 2, acute DZP (2 mg/kg) induced effects 24 h later, possibly secondary to withdrawal. In a nonsocial context (Experiment 3), DZP increased exploratory activity.

Keywords: Diazepam; Social stress; Anxiety; Defeat; DBA/2 mice; Benzodiazepines; Mice; Risk assessment; Flight

1. Introduction

Social stress induces marked physiological changes including increased activity of the sympathetic nervous system and of the hypothalamic-pituitary axis, and has pervasive behavioral effects (reviewed in [7,33,43]). Social defeat (SD) has been used as an animal model of depression [33], which is often a symptom of, or comorbid with, anxiety disorders [49]. Some social stress-induced behaviors may be analogous to symptoms of anxiety disorders, such as exaggerated avoidance often seen in post-traumatic stress disorder (PTSD; [47]). In the diverse class of clinical anxiety disorders, several symptoms are not responsive to the benzodiazepines (BZs). Symptoms of panic disorder, PTSD, obsessive–compulsive disorder and specific phobias, all of which are classified as anxiety disorders in the DSM-IV [14] and are associated with fear and autonomic arousal, typically are not responsive to standard BZ treatment (reviewed in Refs. [26,46]). The potential utility of ethological models in the study of preclinical pharmacology of anxiety disorders has recently received increased consideration [5].

We used a modified resident–intruder test to develop a comprehensive ethogram of behavioral changes following SD in male DBA/2 mice and tested their responses to acute diazepam (DZP) exposure. In the resident–intruder test, a resident mouse usually approaches and attacks an intruder placed within its cage (reviewed in Ref. [38]). After SD, resident mice display fewer approaches and greater avoidance, defense, and active flights than do nondefeated (NOSD) mice, even in response to nonaggressive intruders (NAIs) [30,43,44]. In addition to these measures, we pre-

☆ Intramural research was funded by U.S. Army Medical Research and Material Command. The views of the authors do not purport to reflect the position of the Dept. of the Army or the Dept. of Defense (par. 4-3), AR 360-5.

* Corresponding author. Tel.: +1-301-319-9307; fax: +1-301-319-9905.

E-mail address: lucille.lumley@na.amedd.army.mil (L.A. Lumley).

0091-3057/00/$ – see front matter
PII: S0091-3057(00)00382-8

sently report on the effects of SD on risk assessment. Although measures of risk assessment including stretched-approach and stretched-attend posture (SAP) have typically been measured in nonsocial contexts (reviewed in Refs. [15,42]), these behaviors have also been measured in response to a predator's odor [2,24] and a "stretched posture" in mice confronting a conspecific has been described [25]. We currently use the term stretched-approach to refer to slow approach of the subject towards the intruder, with its stretched body lowered to the ground. We use the term SAP to refer to when the subject's neck is extended, moving its head forward while its hind legs are positioned together on the ground. These behaviors appear to be investigative, yet cautious in nature. We used three subtests, each with increasing threat exposure, to broaden our ethological analysis of agonistic behavior.

DBA/2 mice are considered to have an intermediate level of anxiety relative to other mouse strains, based on performance in the elevated plus maze, open field test and light–dark test [11,51]. In most animal models of anxiety, including tests of conflict, social interaction, exploratory behavior, fear-potentiated startle and stress-induced vocalizations, BZs are anxiolytic (reviewed in Refs. [21,32,42]). However, depending on the context, dose, treatment regimen, particular BZ, route of administration, and prior experience, BZs may induce enhanced flight behavior (reviewed in Ref. [15]). In resident hamsters, acute DZP exacerbated flight from an NAI in a generalization test 24 h after SD, both in hamsters that received DZP immediately after SD, and in those that received DZP prior to the test [28]. Dixon and Kaesermann [15] reviewed animal studies in which DZP induced approach and flight and suggested that this behavioral disinhibition might be analogous to "paradoxical aggression" sometimes elicited by anxiolytics in humans. Low doses of DZP can increase aggressive behavior, while high doses inhibit it (reviewed in Ref. [39]). Acute DZP exposure increased aggressive behavior in humans with low-anxious individuals responding more aggressively than high-anxious individuals [54]. During SD, DBA/2 mice are highly reactive [44]. These mice continue to display escape attempts following repeated defeats, while C57BL/6 mice inhibit escape attempts [36]. In addition, DBA/2 mice did not display anxiolytic responses to BZ when tested in the light–dark test [11]. We hypothesized that during an agonistic encounter, this mouse strain would display enhanced flight or reactivity to low doses of DZP, rather than aggressive behavior.

Pharmacological agents, including BZs, often affect socially stressed animals differently than they do nonstressed animals [52]. A variety of stressors, including swim, handling, noise and tail pinch affect the GABA/BZ complex; the nature and direction of effects are dependent on the type of the stressor (reviewed in Ref. [19]). Stress may also modify the effect of DZP through the induction of DZP binding inhibitor [48]. Stress effects on the pharmacokinetics of DZP may affect further stress responses. Social stress in mice increases BZ receptors, an effect dependent on adrenal integrity [40]. A stressful event remote in time might alter the effects of DZP. Antelman et al. [1] reported that a single episode of restraint stress in rats was shown to block the effects of DZP one month later. We hypothesized that mice that received SD prior to DZP exposure would respond differently than nonstressed mice.

Goals of the current experiments included (1) the expansion of our ethological analysis of social-stress-induced behavioral changes in DBA/2 mice, (2) the determination of how this "intermediate anxious" mouse strain would respond under the influence of, and following withdrawal from, DZP, and (3) the determination of whether previous social stress would affect the responsiveness of DBA/2 mice to DZP during an agonistic encounter. In Experiment 1, resident subject mice received DZP prior to the resident–intruder test, 24 h after SD or NOSD, in order to test whether DZP would affect behavior in this generalization test. Since DZP has amnesic effects [41], in Experiment 2 we tested whether DZP administered immediately after SD would block acquisition of defeat-induced behavioral changes tested 24 h later. In Experiment 3, we tested whether DZP would affect exploratory activity in a nonsocial situation, in order to determine whether DZP effects on activity levels are context-dependent. These experiments provide additional measures of social stress induced behavioral changes and provide further support that acute DZP exposure may have paradoxical effects depending on the social context.

2. Materials and methods

Research was conducted in compliance with the Animal Care Welfare Act, and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles in the Guide for Care and Use of Laboratory Animals, National Research Council, National Academy Press, Washington, DC, 1996, and the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985).

2.1. Animals

Adult male DBA/2 mice (20–22 g; Charles River Labs) were housed in reverse 12:12 light–dark cycle (lights off at 0900 h) in a temperature ($20 \pm 3^{\circ}C$)- and humidity ($50 \pm 20\%$)-controlled room, with food and water available ad libitum. Subject mice were housed five per cage until 2 weeks prior to the experiment, at which time they were individually housed in cages with dimensions $48 \times 27 \times 20$ cm.

Aggressive C57BL/6 male mice (25–30 g) were individually housed for over 2 months prior to the experiment, since isolation induces aggressive behavior [8]. Aggressors that had short attack latencies and initiated many attacks within a short period of time were selected and trained with

previously defeated mice. In Experiment 1, C57BL/6 stimulus mice prescreened for use as NAI received a high dose of DZP (20 mg/kg, ip), which inhibits aggressive behavior (reviewed in Ref. [39]). Since the NAI attacked two subject mice in Experiment 1, mice that had received olfactory bulbectomies were used as intruders in Experiment 2. These mice approach the subject, occasionally displaying tail rattles, but do not attack [13].

2.2. Experimental procedures

In Experiment 1, 26 mice that received SD and 27 mice that were not defeated (NOSD), were treated with DZP (0, 0.5, 2.0 mg/kg, ip) 24 h post-defeat or NOSD, 30 min prior to the modified resident–intruder test. In Experiment 2, 28 SD mice and 26 NOSD mice received DZP (0, 0.5, 2.0 mg/kg, ip) immediately after SD or NOSD, and 24 h later were tested in the resident–intruder test. In Experiment 3, 13 mice were counterbalanced to receive DZP (0, 0.5, and 2.0 mg/kg, ip) 30 min prior to a Figure-8 maze test of exploratory and locomotor activity, with 4 days between each test to allow for drug elimination.

2.3. Acute SD

SD consisted of three 2-min pairings in the home cages of three different highly aggressive C57BL/6 mice, with 2-min rest periods between trials. During rest peri-

Table 1
Operational definitions of behaviors measured during the modified resident–intruder test

Aggressive	
Approach	Walking toward barrier or NAI
Attack	Rapid lunge at NAI without contact
Attack bite	Rapid lunge at NAI with contact (subjectively perceived by tester)
Chase (pursuit)	Pursue or follow NAI
Grooming NAI	With teeth, pull on fur of NAI
Offensive posture	Approach NAI followed by upright posture towards NAI
Piloerection	Fur standing on end (subjectively perceived by tester)
Runbacks (aggressive)	Running to cage back followed by rapid reapproach
Defense	
Crouch defense	Hunched posture with forepaws slightly elevated
Defensive attack	Attack in response to NAI approach
Freeze	Sudden arrested movement
Upright immobile	Upright tilted posture not directed at intruder and not a rear
Upright defense	Vertical posture in response to intruder approach
Risk assessment	
Stretched-approach (St-ap)	Slow forward locomotion with body lowered to cage bottom
Stretched-attend posture (SAP)	Elongated body posture with hind paws planted and head fully extended
Sniffing	
Sniffing (general)	General sniffing of environment not directed at NAI or bedding
Sniffing bedding	Sniffing of wood chips that comprise cage bedding
Sniffing NAI	Sniffing of NAI
Escape	
Flight (active)	Rapid retreat, typically followed by immobility or defensive posture
Leap	Vertical active escape attempt with all paws leaving the cage bottom
Wall climb	Upright with all paws rapidly moving against cage side/bottom; escape attempt
Exploratory	
Climb on barrier	Climb on the perforated barrier using all four paws
Digging	Burrowing in cage bedding, typically with forepaws
Locomotor activity	Walking
Nosepoke	Poking nose through cage hole accessible during interaction
Rearing	Vertical elevation of forepaws not in direct response to NAI (above 45° angle)
Miscellaneous	
Barrier avoidance	Duration in the back half of the cage
Body twitch	Rapid body shake
Chew bedding	Chewing on wood chips that comprise cage bedding
Ear wiggling	Rapid vibratory movement of the ears
Grooming (self)	Auto-grooming including licking and scratching of body and head
Sitting	Crouched posture with all paws on ground
Straub tail	Stiffening of tail musculature with 45° angle above body

ods, a barrier separated the subject from the next aggressor. The number of attacks and bites were recorded using a handheld counter. SD was operationally defined for these studies as three defeat trials, with each trial ending at either 2 min or 35 attacks. Hebert et al. [29] observed that three 2-min defeat trials were needed before defeat-induced swim immobility was observed. We, and others [43], have observed that the number of attacks positively correlates with barrier avoidance 24 h later, and that three 2-min defeat trials induced long-term inhibition of territorial marking [37].

2.4. Drugs

Diazepam, purchased from Sigma Chemical, was suspended in 0.2% Tween in saline, and administered intraperitoneally in a volume of 10 ml/kg. Since 0.5 mg/kg DZP has been observed to be the minimally effective anxiolytic dose [22], this dose was selected as the lowest dose tested.

2.5. Modified resident–intruder test

Subject mice were videotaped in their home cages during three consecutive, 5-min subtests. In the first test (habituation test), a perforated barrier was placed within the home cage of the resident subject to acclimate the subject to the barrier. In the second test (barrier test), an NAI was placed on the other side of the barrier and in the third test (social interaction test) the barrier was removed so mice could directly interact. The computer program Hindsight (Scott Weiss, UK) was used by two observers, blind to the treatment conditions, to score behaviors concurrently. Since some behaviors occurred simultaneously, the summed duration of all behaviors would exceed the total test duration. The frequency and duration of the behaviors scored, many of which were derived from Grant and Mackintosh [25] are indicated (Table 1). Two behaviors, approach and locomotor activity, of the NAI were also scored.

Table 2
Experiment 1: factor analysis results of three subtests

A. Habituation subtest Factors				
Factor 1 (Groom T)	Groom T: 0.70	Groom F: 0.52	Sitting F: 0.33	
Factor 2 (Appr, Rear F)	Appr F: 0.83	Rearing F: 0.88	Loco T: 0.80	Sniff TF: 0.75, 0.36
Factor 3 (Str-app F, SAP T)	Str-app TF; 0.71, 0.80	SAP TF: 0.7, 0.73	Crouch T: 0.47	Crouch F: 0.52
Factor 4 (Climb bar T)	Climb bar T: 0.46	Climb bar F: 0.45		
Factor 5 (Body shake F)	Body shake F: 0.62	Groom F: 0.34		
Factor 6 (Crouch T)	Crouch TF: 0.60	Crouch F: 0.56	Groom TF: 0.53, 0.48	Freeze F: 0.45
Factor 7 (Digging, Sniff bed T)	Digging TF: 0.35, 0.45	Sniffing F: 0.60	Sniff bed T: 0.45	Approach F: 0.40
Factor 8 (Sitting T)	Sitting T: 0.38	Sniffing F: 0.47	Runback F: 0.41	
Barrier avoidance, which did not load high on any factor, was also selected for further analysis.				
B. Barrier subtest Factors				
Factor 1 (Crouch T, Avoid T, Str-app F)	Crouch TF: 0.72, 0.70	Avoid: 0.59	Str-app TF: 0.50, 0.56	SAP TF: 0.48, 0.65
Factor 2 (Groom T)	Groom TF: 0.71, 0.65	Body shake F: 0.70	Str-app TF: 0.49, 0.46	Chew bed F: 0.46
Factor 3 (Appr, Flight, Rearing F)	Appr F: 0.86	Sniff NAI TF: 0.56, 0.75	Flight F: 0.75	Rearing: 0.61
Factor 4 (Digging, Loco T)	Dig TF: 0.60, 0.59	Loco T: 0.48	Sniff bed F: 0.39	Sniff bed F: 0.38
Factor 5 (SAP T)	SAP TF: 0.52, 0.38	Sniffing T: 0.52	Dig TF: 0.41, 0.43	Sniff bed F: 0.44
Factor 6 (Sniff bed T)	Sniff bed T: 0.40	Sniff bed F: 0.38		
Factor 7 (Straub T)	Straub TF: 0.49	Straub F: 0.51	Climb bar T: 0.40	Climb bar F: 0.36
C. Social interaction subtest Factors				
Factor 1 (Attack F, Chase F, Loco T)	Attack F: 0.80	Chase F: 0.76	Groom NAI TF: 0.73	Loco T: 0.70
Factor 2 (SAP T, Str-app F)	SAP TF: 0.72, 0.65	Sniff bed TF: 0.71, 0.57	Sniffing TF: 0.66, 0.68	Str-app TF: 0.36, 0.50
Factor 3 (Upright def F)	Upright def F: 0.46	Sitting F: 0.43	Wall climb F: 0.31	
Factor 4 (Approach F)	Approach F: 0.4	Sniff NAI: 0.34, 0.47	Straub TF: 0.43, 0.46	Flight F: 0.35
Factor 5 (Sniff bed T)	Sniff bed T: 0.31	Sitting F: 0.51	Sitting T: 0.41	
Factor 6 (Leap F)	Leaps F: 0.46	Wall climb F: 0.45	Ear wiggling: 0.4	Upright imm F: 0.35
Factor 7 (Straub T)	Digging TF: 0.50, 0.49	Straub F: 0.44	Straub T: 0.39	Nosepoke TF: 0.37, 0.30
Factor 8 (Pilerection T)	Off post F: 0.35	Piloerection T: 0.34		
Behaviors selected for analysis that did not load high on any component included rears, crouch and groom.				

Column 1 represents the behaviors selected to represent that component. Each row represents separate components, with factor loadings indicated.

T = time; F = frequency; Appr = approach; Avoid = avoidance; Chew bed = chew bedding; Climb bar = climb barrier; Crouch = crouch defense; Groom = self-grooming; Loco = locomotor activity; Off post = offensive posture; SAP = stretched-attend posture; Sniffing bed = sniff bedding; Str-app = stretched-approach; Straub = Straub tail; Upr def = upright defensive posture; Upr imm = upright immobility.

2.6. Exploration and locomotor activity in Figure-8 maze

In Experiment 3, mice received DZP (0, 0.5, 2.0 mg/kg, ip) 30 min prior to being tested for 10 min for their level of exploratory activity in a Figure-8 maze (San Diego Instruments). The dependent measure was the number of photobeam interruptions.

2.7. Statistical analysis

The statistical package SPSS 9.0 (SPSS) was used. For Experiments 1 and 2, behavioral measurements to be analyzed using a two-way analysis of variance (ANOVA), with defeat status and drug dose as factors, were selected based on exploratory results from factor analysis (Principle Components Extraction) using correlation coefficients. Since the types of behaviors expressed varied across subtests, factor analysis was performed on behaviors measured in each subtest; behaviors that were rarely expressed were excluded from analysis. Behavioral measurement(s) that loaded high on a particular component (see Tables 2, 3) were selected to represent that component in the ANOVA. Significant interaction effects were analyzed using a one-way ANOVA for SD and NOSD mice. In Experiment 3, a one-way ANOVA was performed with drug dose as the independent factor. Significant data were further probed using Dunnet's *t* test. A significance level of $P < .05$ was selected.

3. Results

Overall, SD mice had increased passive and active avoidance, defense, risk assessment, and decreased aggressive behavior in response to the NAI, relative to NOSD

Table 3
Experiment 2: factor analysis results of three subtests

A. Habituation subtest Factors				
Factor 1 (Avoid, Groom T)	Avoid T: 0.83	Sitting FT: 0.83, 0.76	SAP FT: 0.67, 0.67	Groom TF: 0.65, 0.54
Factor 2 (Appr F, Loco T)	Approach F: 0.89	Loco T: 0.82	Runback F: 0.66	Rearing F: 0.53
Factor 3 (Str-app F)	Str-app F: 0.47	Str-app T: 0.41	Climb bar T: 0.41	SAP T: 0.38
Factor 4 (Digging T)	Digging T: 0.58	Digging F: 0.61		
Factor 5 (Body shake F)	Body shake F: 0.73	Crouch F: 0.38	Groom F: 0.37	Runback F: 0.33
Factor 6 (Crouch T)	Crouch TF: 0.57	Crouch F: 0.47	Straub F: 0.42	Straub F: 0.43
Factor 7 (SAP T)	SAP TF: 0.35, 0.34	Crouch T: 0.46	Sniffing F: 0.33	Rearing F: 0.33
Factor 8 (Sitting T)	Sitting T: 0.37			
B. Barrier subtest Factors				
Factor 1 (Str-app F)	Str-app TF: 0.44, 0.43	Sniff bed TF: 0.5, 0.4	Sniffing F: 0.40	Digging F: 0.35
Factor 2 (Groom T)	Groom TF: 0.31, 0.38	Str-app TF: 0.45, 0.37	Body shake F: 0.38	Climb bar T: 0.36
Factor 3 (Appr, Flight F)	Appr F: 0.79	Straub TF: 0.60, 0.69	Flight F: 0.62	Sniff NAI F: 0.36
Factor 4 (Rearing F)	Rearing F: 0.68	Sniff NAI TF: 0.71, 0.67	Loco T: 0.62	Runback F: 0.70
Factor 5 (Loco T)	Digging F: 0.54	Digging T: 0.46	Loco T: 0.41	
Factor 6 (Sniff bed T)	Sniff bed F: 0.42	Sniff bed F: 0.40	Digging T: 0.37	
Factor 7 (Straub T)	Straub T: 0.57	Straub F: 0.56		
Factor 8 (Ear wiggling F)	Ear wiggling F: 0.58	Sniff bed TF: 0.48, 0.50	Freeze F: 0.45	Groom TF: 0.45, 0.34
Avoidance, flight, SAP and crouch defense did not load high on any components, but were selected for further analysis based on a priori expectations and previous experiments.				
C. Social interaction subtest Factors				
Factor 1 (Appr, chase F)	Approach F: 0.77	Sniff NAI TF: 0.7, 0.69	Chase F: 0.60	Runback F: 0.60
Factor 2 (Groom T)	Body shake F: 0.68	Groom T: 0.58	Groom F: 0.67	
Factor 3 (SAPT, Str-ap F)	SAP TF: 0.60, 0.68	Str-app F: 0.52	Str-app F: 0.52	Sniffing: 0.50, 0.66
Factor 4 (Leap F)	Upright def F: 0.55	Leap F: 0.41	Sniff NAI T: 0.50	Sniffing NAI F: 0.47
Factor 5 (Straub T)	Straub T: 0.41	Straub F: 0.41		
Factor 6 (Loco T, Rearing F)	Rearing F 0.54	Loco F: 0.52	Nosepoke F: 0.52	Dig TF: 0.43, 0.39
Factor 7 (Piloerection T)	Piloerection T: 0.41			
Factor 8 (Ear wiggle F)	Ear wiggling F: 0.34			
Factor 9 (Upright imm F)	Upright imm F: 0.51	Chew Bed F: 0.45		
Behaviors selected for analysis that did not load high on any component include crouch and flight.				

Column 1 represents the behavioral measurement(s) selected to represent that component. Each row represents separate components, with factor loadings indicated.

T = time; F = frequency; Appr = approach; Avoid = avoidance; Chew bed = chew bedding; Climb bar = climb barrier; Crouch = crouch defense; Groom = self-grooming; Loco = locomotor activity; Off post = offensive posture; SAP = stretched-attend posture; Sniffing bed = sniff bedding; Str-appr = stretched-approach; Straub = Straub tail; Upright def = upright defensive posture; Upright imm = upright immobility.

mice. In Experiment 1, 0.5 mg/kg DZP increased approach and flight, whereas 2.0 mg/kg DZP increased passive avoidance and decreased risk assessment. In Experiment 2, a few effects of prior exposure to DZP were observed. Means and standard errors ($x \pm$ S.E.) are included in Table 4.

The following behaviors were selected for analysis for each subtest using factor analysis (T = time; F = frequency; see factor analysis tables, Tables 2 and 3).

Habituation subtest: barrier avoidance (T), crouch defense (T), stretched-approach (F), SAP (T), sniffing bedding (T), approach (F), rearing (F), locomotor activity (T), digging (T), grooming (T). Rarely expressed and omitted from analysis were: flights, leaps, upright immobility, piloerection, wall climbing, ear wiggling, chew bedding, Straub tail and behaviors that require the presence of another animal (e.g., attack).

Barrier subtest: barrier avoidance (T), crouch defense (T), stretched-approach (F), SAP (T), sniffing bedding (T), approach (F), rearing (F), flight (F), locomotor activity (T), grooming (T), Straub tail (T). Sniffing bedding was rarely expressed in Experiment 2 and ear wiggling (F) rarely in Experiment 1; therefore, in Experiment 2 ear wiggling was analyzed instead of sniffing bedding. Behaviors omitted from analysis were: leaps, upright immobility, wall climbing, tail rattle, chew cage bedding and those requiring contact (e.g., attack).

Social interaction subtest: stretch approach (F), SAP (T), crouch defense (T), approach (F), rearing (F), chase (F), attack (F), flight (F), leap (F), locomotor activity (T), grooming (T), Straub tail (T), ear wiggling (F). Behaviors rarely expressed and omitted were: circle, defensive attack, sitting, tail rattle, grooming NAI.

Table 4
T = duration (s); F = frequency; SAP = stretched-attend posture.

	NOSD ($x \pm$ S.E.M.)			SD ($x \pm$ S.E.M.)		
Behaviors	0 mg/kg DZP	0.5 mg/kg DZP	2.0 mg/kg DZP	0 mg/kg DZP	0.5 mg/kg DZP	2.0 mg/kg DZP
A. Experiment 1: behaviors measured during the habituation subtest in DBA/2 mice treated with DZP (0, 0.5, 2.0 mg/kg, ip) 30 min prior to the test						
Avoidance (T)	81.20 ± 24.46	86.76 ± 26.05	222.84 ± 29.12 $^{+}$	128.65 ± 29.12	100.93 ± 27.45	209.31 ± 27.46 $^{+}$
Crouch defense (T)	0.73 ± 9.34	3.21 ± 8.86	0.29 ± 9.91	4.18 ± 9.91	3.15 ± 9.34	21.96 ± 9.34
Stretch-approach (F)	0.11 ± 0.37	0.40 ± 0.35	0.50 ± 0.39	1.00 ± 0.39	0.78 ± 0.37	0.67 ± 0.37
SAP (T)	11.04 ± 5.10	16.77 ± 4.84	15.72 ± 5.41	15.75 ± 5.41	16.78 ± 5.10	14.90 ± 5.10
Sniff bedding (T)	1.00 ± 0.66	1.40 ± 0.62	2.63 ± 0.69 $^{+}$	1.63 ± 0.69	1.89 ± 0.66	3.44 ± 0.66 $^{+}$
Approach (F)	9.00 ± 1.20	7.60 ± 1.14	2.13 ± 1.28 $^{+}$	4.38 ± 1.27	8.89 ± 1.20 $^{\#}$	3.22 ± 1.20 $^{+}$
Rearing (F)	23.67 ± 3.31	26.90 ± 3.14	10.88 ± 3.51 $^{++}$	20.88 ± 3.51	24.22 ± 3.31	12.33 ± 3.31 $^{++}$
Locomotion (T)	72.31 ± 9.21	82.55 ± 8.74 $^{+}$	40.47 ± 9.77 $^{+}$	61.04 ± 9.77	95.04 ± 9.21 $^{+}$	46.57 ± 9.21 $^{+}$
Digging (T)	16.76 ± 12.85	5.11 ± 12.19	18.67 ± 13.63	14.09 ± 13.63	11.10 ± 12.85	58.38 ± 12.85
Grooming (T)	9.66 ± 4.18	11.14 ± 3.97	10.21 ± 4.43	18.54 ± 4.43	11.84 ± 4.18	8.89 ± 4.18
B. Experiment 1: behaviors measured during the barrier subtest test in DBA/2 mice treated with DZP (0, 0.5, 2.0 mg/kg, ip) 30 min prior to the test						
Crouch defense (T)	57.25 ± 19.54	68.69 ± 18.53	99.77 ± 20.72	113.73 ± 20.72	97.38 ± 19.54	95.66 ± 19.54
Stretch-approach (F)	1.22 ± 0.41	0.80 ± 0.39	0.63 ± 0.44	0.75 ± 0.44	1.44 ± 0.41	0.22 ± 0.41
SAP (T)	22.68 ± 5.63	18.18 ± 5.34	15.19 ± 5.97 $^{+}$	22.24 ± 5.97	27.13 ± 5.63	8.54 ± 5.63 $^{+}$
Sniff bedding (T)	2.39 ± 13.59	2.14 ± 12.89	34.55 ± 14.41 $^{+++}$	2.95 ± 14.41	6.31 ± 13.59	78.33 ± 13.59 $^{+++}$
Rearing (F)	12.11 ± 3.63	19.50 ± 3.44 $^{+}$	7.62 ± 3.85	9.75 ± 3.85	18.00 ± 3.63 $^{+}$	6.67 ± 3.63
Locomotion (T)	37.66 ± 7.11	37.83 ± 6.75	36.50 ± 7.54	21.70 ± 7.54	43.79 ± 7.11	32.25 ± 7.11
Grooming (T)	11.08 ± 5.37	6.16 ± 5.10	2.75 ± 5.70 $^{++}$	26.75 ± 5.70	16.40 ± 5.37	2.16 ± 5.37 $^{++}$
C. Experiment 1: behaviors measured during direct social interaction in DBA/2 mice treated with DZP (0, 0.5, 2.0 mg/kg, ip) 30 min prior to the test						
Crouch defense (T)	67.35 ± 14.23	81.90 ± 13.50	85.27 ± 15.10	96.40 ± 15.10	102.97 ± 14.23	78.54 ± 14.23
Stretched-approach (F)	0.67 ± 0.19	0.10 ± 0.18	0.00 ± 0.20 $^{+}$	0.50 ± 0.20	0.22 ± 0.19	0.11 ± 0.19 $^{+}$
SAP (T)	8.70 ± 2.9	4.41 ± 2.79	5.86 ± 3.12	12.56 ± 3.12	4.09 ± 2.94	3.62 ± 2.94
Approach (F) **	8.44 ± 2.05	12.20 ± 1.95	10.38 ± 2.18	4.63 ± 2.18	4.67 ± 2.05	8.22 ± 2.05
Rearing (F) *	12.33 ± 3.21	18.60 ± 3.04	21.00 ± 3.04 $^{++}$	16.75 ± 3.40	23.44 ± 3.21	29.33 ± 3.21 $^{++}$
Chase (F)	1.00 ± 0.30	0.10 ± 0.28	0.00 ± 0.31	0.00 ± 0.31	0.00 ± 0.30	0.00 ± 0.30
Attack (F)	4.11 ± 1.46	0.30 ± 1.39	0.13 ± 1.55	0.00 ± 1.55	0.00 ± 1.46	0.00 ± 1.46
Leap (F)	0.67 ± 2.85	7.00 ± 2.70	6.63 ± 3.02	9.25 ± 3.02	6.56 ± 2.85	10.44 ± 2.85
Locomotion (T) *	75.30 ± 8.25	66.94 ± 7.83	63.18 ± 8.75	57.01 ± 8.75	51.75 ± 8.25	50.12 ± 8.25
Grooming (T)	1.86 ± 1.80	5.80 ± 1.71	0.12 ± 1.91	1.98 ± 1.91	2.77 ± 1.80	1.60 ± 1.80
Straub tail (T)	39.83 ± 9.78	11.10 ± 9.28	5.52 ± 10.37 $^{++}$	37.38 ± 10.37	36.08 ± 9.78	0.43 ± 9.78 $^{++}$
NAI locomotion (T)	232.51 ± 22.66	256.57 ± 21.50	245.71 ± 24.04	247.46 ± 24.04	238.59 ± 22.66	232.43 ± 22.66
NAI approach	17.67 ± 3.44	23.20 ± 3.26	20.38 ± 3.65	23.88 ± 3.65	23.78 ± 3.44	18.22 ± 3.44

* SD effects: $P < .05$.
** SD effects: $P < .01$.
$^{+}$ DZP effects: $P < .05$.
$^{++}$ DZP effects: $P < .01$.
$^{+++}$ DZP effects: $P < .001$.
$^{\#}$ Interaction effects: $P < .05$.

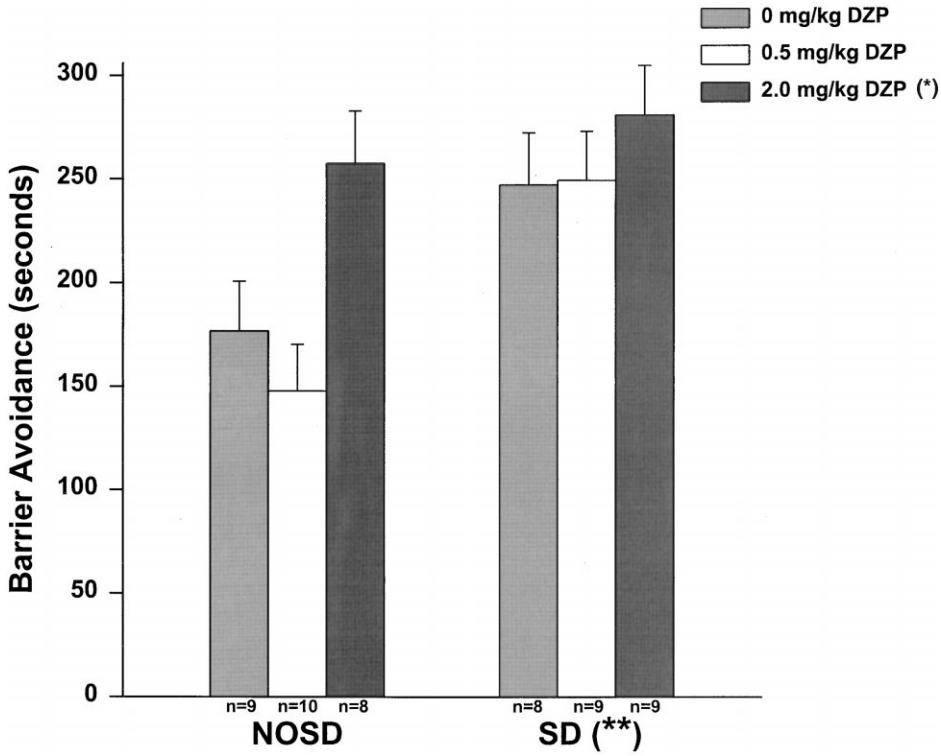

Fig. 1. During the barrier subtest of Experiment 1, DBA/2 mice that received social defeat (SD) displayed more barrier avoidance than mice that were not defeated (NOSD; ** $P < .01$). In addition, mice that received acute administration of 2.0 mg/kg diazepam (DZP) had more avoidance than vehicle-treated mice, independent of social defeat (* $P < .02$).

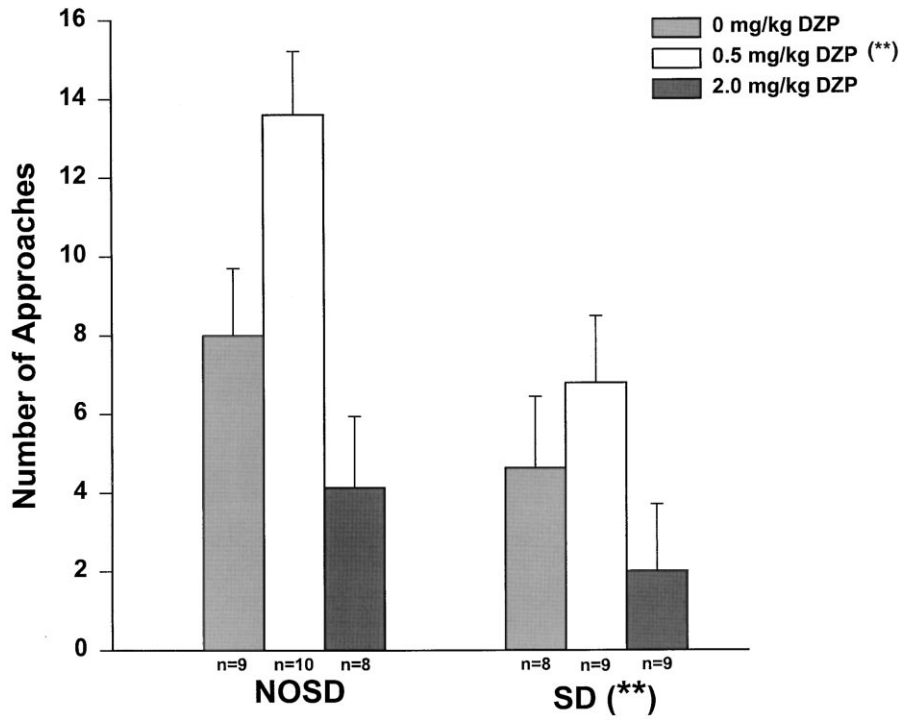

Fig. 2. During the barrier subtest of Experiment 1, DBA/2 mice that received social defeat (SD) displayed fewer approaches than mice that were not defeated (NOSD; ** $P < .01$). In addition, mice that received 0.5 mg/kg diazepam (DZP) displayed more approaches than vehicle-treated mice, independent of social defeat (** $P < .01$).

3.1. Experiment 1

Means ± standard error are indicated in Table 4.

3.1.1. Habituation subtest (see Table 4A)

3.1.1.1. Investigative behaviors. There were no effects of SD. Locomotor activity was increased in mice that received 0.5 mg/kg DZP but decreased in mice that received 2.0 mg/kg DZP, relative to VEH [$F(2, 52) = 12.01$]. In addition, 2.0 mg/kg DZP inhibited rearing [$F(2, 52) = 9.45$] and approaches [$F(2, 52) = 11.17$], while increasing the duration of sniffing bedding, relative to VEH [$F(2, 52) = 8.45$]. In SD mice, 0.5 mg/kg DZP (8.89 ± 2.45) increased approaches relative to VEH (4.38 ± 3.74; $F(2, 52) = 3.75$).

3.1.2. Barrier subtest (see Table 4B)

3.1.2.1. Avoidance. SD mice had increased passive avoidance, demonstrated by a greater duration of barrier avoidance [$F(1, 52) = 11.02$; Fig. 1] and fewer approaches [$F(1, 52) = 8.58$; Fig. 2]. Mice, SD as well as NOSD, treated with 0.5 mg/kg DZP had more approaches [$F(2, 52) = 8.83$; Fig. 2] and rears [$F(2, 52) = 5.36$], but also more flights [$F(2, 52) = 4.33$; Fig. 3] than VEH. Mice, SD as well as NOSD, that received 2.0 mg/kg DZP had a greater duration of sniffing bedding [$F(2, 52) = 9.72$] and avoidance [$F(2, 52) = 4.86$; Fig. 1] than had VEH mice.

3.1.2.2. Defensive behavior. SD mice had a nonsignificant trend for a greater duration of crouch defense than NOSD mice.

3.1.2.3. Risk assessment. Mice that received 2.0 mg/kg DZP had a shorter duration of SAP [$F(2, 52) = 2.65$] than VEH.

3.1.2.4. Grooming. SD mice had a nonsignificant trend for more grooming than NOSD mice. Mice that received 2.0 mg/kg DZP groomed less than VEH mice [$F(2, 52) = 4.43$].

3.1.3. Social interaction subtest (see Table 4C)

3.1.3.1. Avoidance. SD mice had fewer approaches [$F(1, 52) = 7.05$] and more rears [$F(1, 52) = 4.90$] than NOSD mice. Mice that received 2.0 mg/kg DZP had more rearing than VEH [$F(2, 52) = 5.26$], possibly indicating escape attempts.

3.1.3.2. Defensive behavior. There was a nonsignificant trend for SD mice to have more crouch defense than NOSD mice.

3.1.3.3. Risk assessment. Mice that received 2.0 mg/kg DZP had fewer stretched-approaches [$F(2, 52) = 4.08$] than VEH.

3.1.3.4. Aggressive and investigative behavior. NOSD mice had a greater duration of locomotor activity [$F(1,$

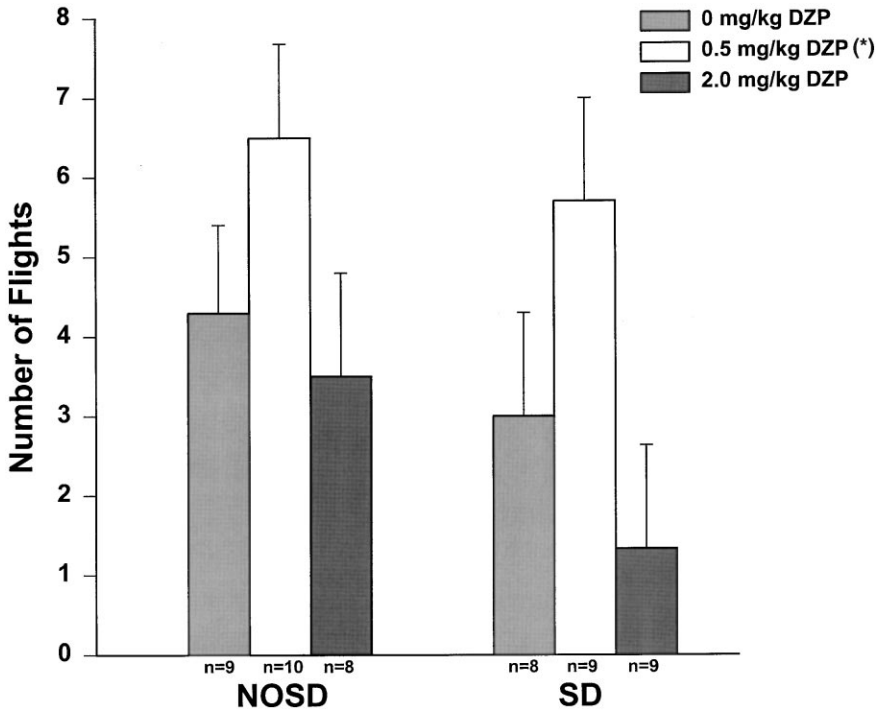

Fig. 3. During the barrier subtest of Experiment 1, DBA/2 mice that received 0.5 mg/kg diazepam (DZP) displayed more flight responses than vehicle-treated mice, independent of social defeat (SD; * $P < .05$). NOSD = nondefeated mice.

52) = 5.17] than SD mice. Four NOSD mice, but no SD mice chased and attacked.

3.1.3.5. Straub tail. Mice that received 2.0 mg/kg DZP had a shorter duration of Straub tail than VEH [$F(2, 52) = 6.30$].

3.2. Experiment 2

3.2.1. Habituation subtest (see Table 5A)

3.2.1.1. Avoidance. SD mice had a greater duration of barrier avoidance than NOSD mice [$F(1, 53) = 4.18$] (Table 5).

3.2.1.2. Investigative behaviors. SD mice had a shorter duration of digging behavior [$F(1, 53) = 9.65$] and fewer rearing [$F(1, 53) = 6.43$] than NOSD mice. Mice that received 2.0 mg/kg DZP 24 h prior to the resident–intruder test had a greater duration of locomotor activity [$F(2, 53) = 5.46$], as well as more approaches [$F(2, 53) = 15.64$] and rearing [$F(2,53) = 3.76$] than VEH. NOSD mice that received 2.0 mg/kg DZP displayed less digging behavior than NOSD mice that received VEH [$F(2, 53) = 4.2$].

3.2.1.3. Grooming. SD mice had a nonsignificant trend for greater duration of grooming than NOSD mice.

Table 5
T = duration (s); F = frequency; SAP = stretched-attend posture.

	NOSD ($x \pm$ S.E.M.)			SD ($x \pm$ S.E.M.)		
Behaviors	0 mg/kg DZP	0.5 mg/kg DZP	2.0 mg/kg DZl	0 mg/kg DZP	0.5 mg/kg DZP	2.0 mg/kg DZP
A. Experiment 2: behaviors measured during the habituation subtest in DBA/2 mice treated with DZP (0, 0.5, 2.0 mg/kg, ip) immediately after SD or NOSD						
Avoidance (T) *	33.32 ± 15.95	40.96 ± 15.95	62.46 ± 15.95	77.31 ± 15.04	69.08 ± 15.04	67.37 ± 14.27
Stretched-approach (F)	0.25 ± 0.37	0.63 ± 0.37	0.50 ± 0.37	1.67 ± 0.35	0.78 ± 0.35	0.50 ± 0.33
SAP (T)	2.20 ± 2.40	2.55 ± 2.40	2.14 ± 2.40	7.07 ± 2.26	2.86 ± 2.26	2.71 ± 2.15
Sniff bedding (T)	15.69 ± 4.28	8.89 ± 4.28	12.90 ± 4.28	12.82 ± 4.04	13.44 ± 4.04	9.74 ± 3.83
Approach (F)	5.88 ± 1.12	6.13 ± 1.12	10.00 ± 1.12 $^{++}$	5.44 ± 1.05	7.33 ± 1.05	12.60 ± 1.00 $^{++}$
Rearing (F) *	26.38 ± 2.62	25.25 ± 2.62	34.00 ± 2.62 $^{+}$	20.11 ± 2.47	24.22 ± 2.47	25.60 ± 2.34 $^{+}$
Locomotion (T)	68.77 ± 6.24	65.10 ± 6.24	80.38 ± 6.24 $^{++}$	69.88 ± 5.89	75.36 ± 5.89	93.31 ± 5.59 $^{++}$
Digging (T) **	25.75 ± 4.53	10.65 ± 4.53	1.19 ± 4.53 $^{\#}$	0.99 ± 4.27	2.08 ± 4.27	1.32 ± 4.05
Grooming (T)	8.99 ± 5.59	9.74 ± 5.59	2.27 ± 5.59	26.63 ± 5.27	7.52 ± 5.27	13.40 ± 5.00
B. Experiment 2: behaviors measured during the barrier subtest in mice treated with DZP (0, 0.5, 2.0 mg/kg, ip) immediately after SD or NOSD						
Crouch defense (T)*, **	15.21 ± 15.46	21.57 ± 15.46	57.19 ± 16.40 $^{+}$	161.42 ± 15.46	137.25 ± 15.46	157.06 ± 14.67 $^{+}$
Stretched-approach (F)*, **	0.22 ± 0.34	0.33 ± 0.34	0.75 ± 0.36	1.44 ± 0.34	2.00 ± 0.34	0.70 ± 0.32 $^{\#}$
SAP (T)*, **	7.23 ± 4.15	7.49 ± 4.15	17.44 ± 4.40	31.96 ± 4.15	25.22 ± 4.15	23.94 ± 3.94
Approach (F)	9.33 ± 1.94	10.44 ± 1.94	12.25 ± 2.06	8.44 ± 1.94	8.78 ± 1.94	6.50 ± 1.84
Rearing (F)*, **	17.89 ± 2.49	17.67 ± 2.49	17.75 ± 2.64	9.11 ± 2.49	10.22 ± 2.49	8.60 ± 2.36
Flight (F) **	1.00 ± 1.12	2.11 ± 1.12	4.50 ± 1.18	5.22 ± 1.12	6.00 ± 1.12	5.30 ± 1.06
Locomotion (T)*, **	69.87 ± 6.77	64.53 ± 6.77	55.91 ± 7.18	32.11 ± 6.77	43.60 ± 6.77	42.30 ± 6.42
Grooming (T) **	5.41 ± 3.48	3.47 ± 3.48	7.25 ± 3.69	10.61 ± 3.48	12.70 ± 3.48	23.77 ± 3.30
Straub tail (T)	4.17 ± 3.33	1.48 ± 3.33	2.74 ± 3.53	7.93 ± 3.33	1.84 ± 3.33	2.73 ± 3.16
Ear wiggling (F) **	0.22 ± 0.48	0.67 ± 0.48	0.88 ± 0.51 $^{+}$	0.89 ± 0.48	2.00 ± 0.48	2.80 ± 0.46
3C. Experiment 2: behaviors measured during direct social interaction in DBA/2 mice treated with DZP (0, 0.5, 2.0 mg/kg, ip) immediately after SD or NOSD						
Crouch defense (T)***	9.65 ± 11.31	20.86 ± 11.31	36.43 ± 12.00 $^{\#}$	147.34 ± 11.39	116.07 ± 11.31	122.84 ± 10.73
Stretch-approach (F)	0.67 ± 0.42	0.89 ± 0.42	1.00 ± 0.45	0.56 ± 0.42	0.22 ± 0.42	0.90 ± 0.40
SAP (T)***	12.40 ± 2.98	9.14 ± 2.98	23.14 ± 3.16 $^{\#}$	6.73 ± 2.98	6.05 ± 2.98	5.44 ± 2.83
Approach (F)***	17.67 ± 1.82	14.56 ± 1.82	14.38 ± 1.93	5.44 ± 1.82	7.56 ± 1.82	7.00 ± 1.73
Rearing (F)	1.43 ± 2.82	14.00 ± 2.82	20.63 ± 3.00	15.56 ± 2.82	20.78 ± 2.82	18.40 ± 2.68
Chase (F)***	2.33 ± 1.39	8.11 ± 1.39 $^{\#}$	2.00 ± 1.42	0.00 ± 1.39	0.00 ± 1.39	0.00 ± 1.27
Attack (F) **	3.22 ± 1.15	5.33 ± 1.15	0.50 ± 1.22	0.00 ± 1.15	0.00 ± 1.15	0.00 ± 1.09
Leaps (F) ** 0.22 ± 1.25	0.00 ± 1.25	0.13 ± 1.33	2.56 ± 1.25	4.78 ± 1.25	1.60 ± 1.19	
Locomotion (T) **	105.99 ± 8.70	80.51 ± 8.70	91.22 ± 9.23	55.91 ± 8.70	71.66 ± 8.70	79.70 ± 8.26
Grooming (T) *	3.79 ± 3.48	6.36 ± 3.48	0.68 ± 3.69	14.38 ± 3.48	5.69 ± 3.48	10.40 ± 3.30
Straub tail (T)***	4.33 ± 3.81	2.65 ± 3.81	0.62 ± 4.04	13.58 ± 3.81	25.82 ± 3.81 $^{\#}$	25.44 ± 3.62 $^{\#}$
Ear wiggling (F)***	0.00 ± 0.38	0.22 ± 0.38	0.38 ± 0.41	1.89 ± 0.38	1.00 ± 0.38	1.10 ± 0.36
NAI locomotion (T)***	172.87 ± 24.99	183.08 ± 24.99	201.39 ± 26.51	229.16 ± 24.99	263.05 ± 24.99	251.77 ± 23.71
NAI approach (F)***	4.78 ± 1.44	4.22 ± 1.44	4.13 ± 1.53	9.44 ± 1.44	9.33 ± 1.44	8.00 ± 1.37

* SD effects: $P < .05$.
** SD effects: $P < .01$.
*** SD effects: $P < .001$.
$^{+}$ DZP effects: $P < .01$.
$^{++}$ DZP effects: $P < .01$.
$^{\#}$ Interaction effects: $P < .05$.

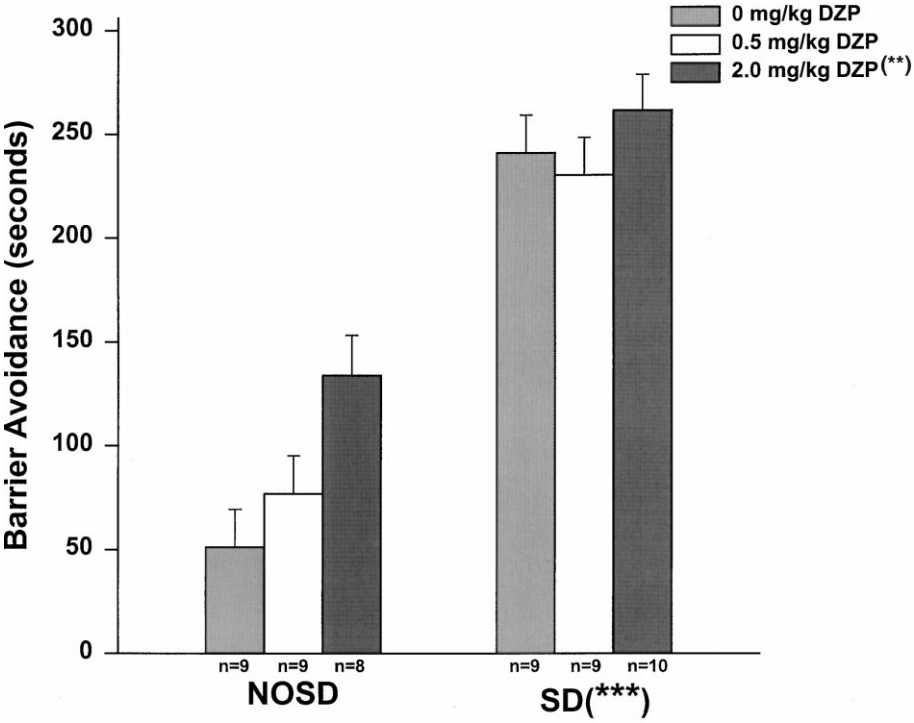

Fig. 4. During the barrier subtest of Experiment 2, DBA/2 mice that received social defeat (SD) displayed more barrier avoidance than those that were not defeated (NOSD; *** $P < .001$). In addition, mice that received 2.0 mg/kg diazepam (DZP) 24 h prior to the generalization test displayed increased avoidance relative to vehicle-treated mice, independent of social defeat (** $P < .01$).

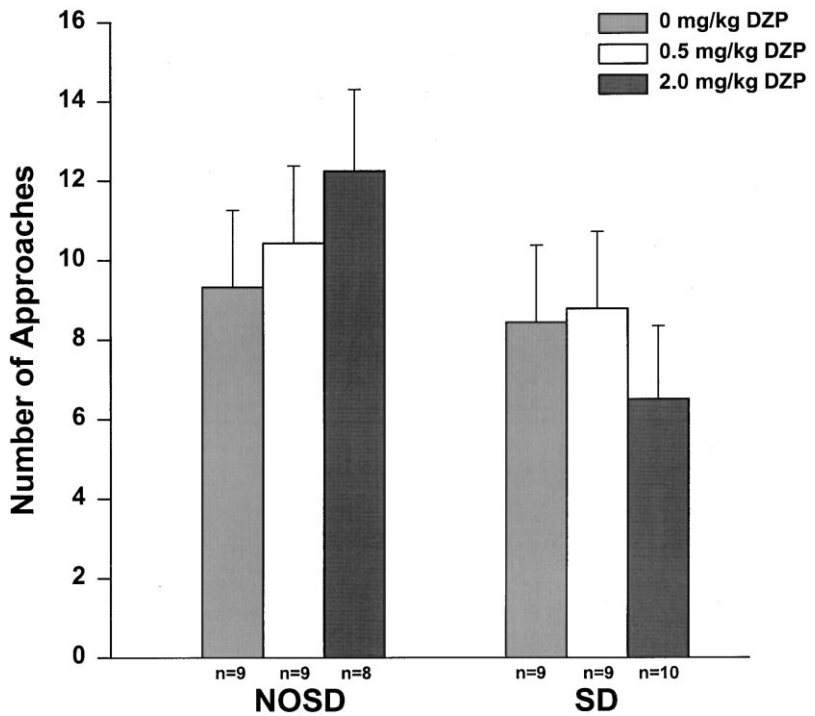

Fig. 5. During the barrier subtest of Experiment 2, there was a nonsignificant trend for mice that received social defeat (SD) to display fewer approaches than mice that were not defeated (NOSD). DZP = diazepam.

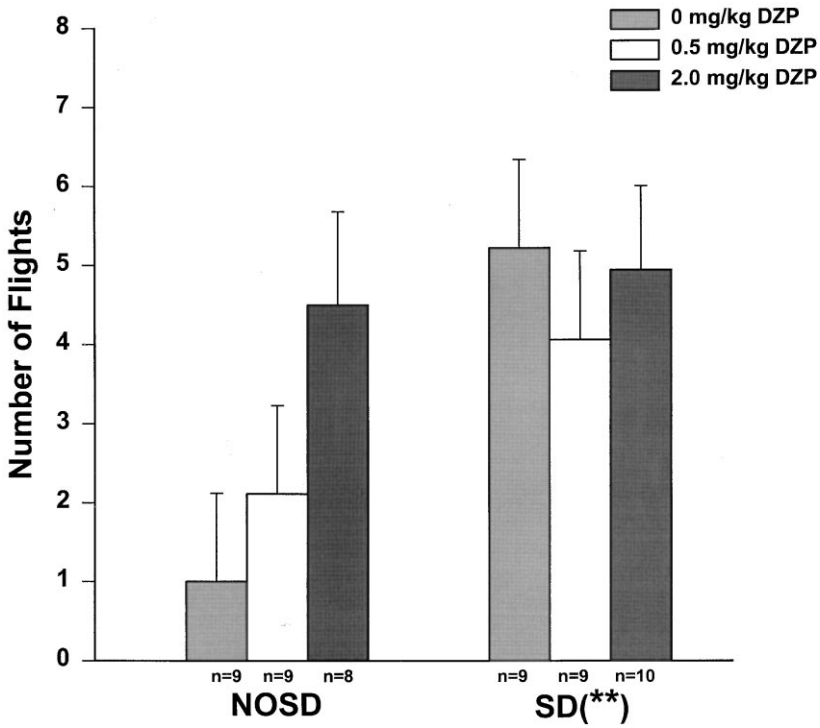

Fig. 6. During the barrier subtest of Experiment 2, mice that received social defeat (SD) displayed more flight responses than mice that were not defeated (NOSD; $**P<.01$). DZP = diazepam.

3.2.2. Barrier subtest (see Table 5B)

3.2.2.1. Avoidance. SD mice displayed passive avoidance, indicated by a greater duration of barrier avoidance [$F(1, 53)=110.7$; Fig. 4] and a nonsignificant trend to have fewer approaches (Fig. 5) than NOSD mice. SD mice displayed active avoidance, indicated by more flights [$F(1, 53)=10.57$; Fig. 6]. Mice, SD as well as NOSD, that received 2.0 mg/kg DZP had a greater duration of barrier avoidance than had VEH mice [$F(2, 53)=4.64$; Fig. 4].

3.2.2.2. Defensive posture. SD mice had a greater duration of crouch defense than had NOSD mice [$F(1,53)=90.87$]. NOSD mice treated with 2.0 mg/kg DZP had a greater duration of crouch defense than had VEH [$F(2, 25)=3.07$].

3.2.2.3. Risk assessment. SD mice had more stretched-approaches [$F(1, 53)=11.7$] and a greater duration of SAP [$F(1, 53)=23.10$] than had NOSD mice.

3.2.2.4. Immobility. SD mice had a shorter duration of locomotor activity [$F(1, 53)=18.92$] and fewer rearing [$F(1, 53)=17.29$] than NOSD mice.

3.2.2.5. Ear wiggling. SD mice had more ear wiggling than had NOSD mice [$F(1, 53)=10.95$]. Mice treated with 2.0 mg/kg DZP had more ear wiggling than had VEH mice [$F(2, 53)=3.55$].

3.2.2.6. Grooming. SD mice had a greater duration of grooming than had NOSD mice [$F(1, 53)=8.45$].

3.2.3. Social interaction subtest (see Table 5C)

3.2.3.1. Avoidance. SD mice had fewer approaches than had NOSD mice [$F(1, 53)=35.36$]. SD mice displayed active avoidance, indicated by more flights [$F(1, 53)=101.98$] and leaps [$F(1, 53)=7.79$] than NOSD mice.

3.2.3.2. Defensive posture. SD mice had a greater duration of crouch defense than NOSD mice [$F(1, 53)=132.33$].

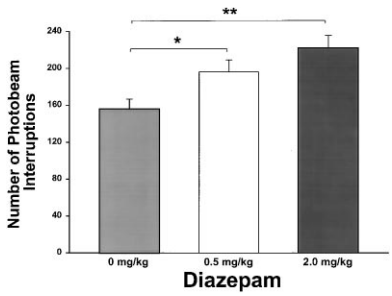

Fig. 7. Diazepam (0.5 mg/kg and 2.0 mg/kg) administered to DBA/2 mice 30 min prior to a Figure-8 test increased locomotor and exploratory activity, relative to vehicle (0 mg/kg). $*P<.05$; $**P<.01$.

3.2.3.3. Risk assessment. SD mice had a shorter duration of SAP than had NOSD mice [$F(1, 53) = 13.08$].

3.2.3.4. Immobility. SD mice had a shorter duration of locomotor activity than had NOSD mice [$F(1, 53) = 10.87$].

3.2.3.5. Aggressive and investigative behaviors. SD mice had fewer chases than had NOSD mice [$F(1, 53) = 14.37$]. NOSD mice that received 0.5 mg/kg DZP had more chases than NOSD mice that received VEH [$F(2, 23) = 3.1$].

3.2.3.6. Ear wiggling. SD mice had more ear wiggling than had NOSD mice [$F(1, 53) = 13.05$].

3.2.3.7. Straub tail. SD mice had a greater duration of Straub tail than had NOSD mice [$F(1, 53) = 37.42$]. In SD mice, there was a nonsignificant trend for 2 mg/kg DZP to increase Straub tail relative to VEH.

3.2.3.8. Grooming. SD mice had a greater duration of grooming than had NOSD mice [$F(1, 53) = 5.29$].

3.2.3.9. OBX NAI behavior. The OBX NAI displayed more approach [$F(1, 53) = 14.85$] and locomotor activity [$F(1, 53) = 9.26$] when paired with SD mice than when paired with NOSD mice.

3.3. Experiment 3: effects of DZP on exploration and locomotor activity

DZP, 0.5 and 2.0 mg/kg, increased exploration and locomotor activity in the Figure-8 maze, relative to VEH [($F(2, 38) = 7.27$; Fig. 7].

4. Discussion

Socially defeated mice displayed more passive and active avoidance, fear responses, and risk assessment, and less exploratory activity, social interaction and aggressive behavior. All three subtests are useful for examining SD-induced behavioral changes, since the subtests produce different, but complementary, patterns of behavior. The habituation test (subtest 1) demonstrates home cage behavior in response to a barrier, a potentially weak reminder of the defeat experience. The barrier test (subtest 2) elicits response to perception of a potential threat, whereas direct social interaction (subtest 3) elicits response to perception of a real threat and elicits more robust fear responses (or attack).

Defeated mice displayed more passive and active avoidance and defense and less exploratory activity. During the habituation test, there were few behavioral effects of SD. In Experiment 2 during habituation, SD mice displayed more barrier avoidance, less locomotor and digging activity and fewer rearing. When an NAI was placed on the opposite side of the barrier, SD mice displayed more barrier avoidance than did NOSD mice. In Experiment 1, SD mice had fewer approaches than did NOSD mice, but there was no difference in the number of flights. In Experiment 2, SD mice had more flights than did NOSD mice, but there was no difference in the number of approaches. Defeated mice typically display more flights and fewer approaches than do NOSD mice [30]. However, if SD mice do not approach the intruder (passive avoidance), then there is no opportunity for flight (active avoidance). In support of this, approach and flight during the barrier test loaded high on components of factor analysis. During social interaction, SD mice had fewer approaches (Experiments 1 and 2) and more flights than had NOSD mice. During the interaction, since the NAI approaches the subject, the subject does not need to approach in order to display flight. Defeated mice had more crouch defense in Experiment 2 (trend Experiment 1) during both the barrier and social interaction subtests. In addition, SD mice had less locomotor activity during both the barrier (Experiment 2) and social interaction subtests (Experiments 1 and 2), and had less sniffing bedding during social interaction than NOSD mice.

SD mice displayed more risk assessment and less aggressive behavior. In Experiment 2, SD mice displayed more SAP and more stretched-approach, measures of risk assessment [42]. Risk assessment is considered to be a less fearful state than flight and escape responses, but a more fearful state than is typically seen in NOSD animals [6]. During social interaction, SAP loaded onto the same component as sniff behavior. In rats, active sniffing behavior is associated with exploration and may be used to increase odor sampling [57], and may be a form of risk assessment [4]. Depending on the context, rearing may also represent risk assessment and exploration [4], or may represent escape attempts (reviewed in Ref. [38]). There were fewer rearing in SD than NOSD mice during the barrier subtest (Experiment 2). This probably indicates decreased exploratory activity during this mildly threatening context. Increased rearing in SD mice during social interaction probably indicated escape attempts in response to a more threatening situation than the barrier subtest. Fewer chases and failure to attack in SD mice indicated decreased social investigation and decreased aggressive behavior, respectively.

Other effects of SD reached significance in Experiment 2, but not Experiment 1. During the barrier and social interaction subtests of Experiment 2 (trend Experiment 1), SD mice had more self-grooming, a measure of anxiety [21] than had NOSD mice. During social interaction, SD mice had more ear wiggling, characterized by a lateral head shaking that produces distinct vibratory movements of the ears (reviewed in Ref. [17]), and more Straub tail, characterized by rigid tail elevation due to contraction of the sacroccoccygeus dorsalis muscle (reviewed in Ref. [27]). One reason for more SD-induced behaviors in Experiment 2 may be differences in the state and behavior of the stimulus mice. In Experiment 1, although the NAI received a high dose of DZP, which inhibits aggressive behavior, the NAI

attacked two subjects and sniffed subjects. Therefore, in Experiment 2, we used an olfactory bulbectomized (OBX) mouse, which inhibits aggressive and sniffing behavior [13], as the NAI; no OBX mice attacked any resident subject in Experiment 2. Anosmic opponents display minimal upright social investigation and elicit different behaviors than do other types of nonaggressive opponents [9]. Fewer effects of SD in Experiment 1 may have resulted from the NAI appearing more threatening than the OBX NAI used in Experiment 2. However, OBX mice displayed more locomotor activity and approach when paired with SD mice than with NOSD mice, which may have affected responses during the social interaction subtest.

Most behavioral effects of acute DZP exposure were independent of defeat status. In Experiment 1, opposite effects of the two doses of DZP were observed: the lower dose induced behavioral disinhibition while the higher dose induced behavioral inhibition. During habituation, mice treated with 0.5 mg/kg DZP had more locomotor activity while during the barrier subtest these mice had more approaches and flights than vehicle-treated mice (VEH). Although flight is often considered a component of defensive behavior [4,42], other measures of defense were not affected by acute DZP. In hamsters, DZP increased flight in those subjected to defeat and tended to increase flight in those that were not defeated [28]. In wild rats, acute BZ administration increased flight in the fear/defense test battery in response to approaching threat of an experimenter, but decreased flight during other aspects of the test indicating that the BZ effects may be context dependent [3]. DZP increased approach and ''weakly'' increased escape attempts (active flights) in other rodent models (reviewed in Ref. [15]). However, in Swiss mice screened for timidity, oral administration of 5 mg/kg DZP increased social and locomotor activity, while reducing defensive behavior and flight [34]. In the light–dark test, DZP increased transitions between light and dark [11], similar to the current findings of DZP (0.5 mg/kg)-induced transitions between approach and flight. Mice that received 2.0 mg/kg DZP displayed less locomotor activity and fewer rearing and approaches and increased sniffing bedding during habituation. During the barrier subtest, these mice displayed barrier avoidance and sniffing bedding and had decreased risk assessment and self-grooming. In a nonsocial context in mice (reviewed in Ref. [15]) and rats [10], DZP decreased SAP. In the current experiments, behavioral inhibition during the first two subtests was not due to sedation since 2.0 mg/kg DZP increased rearing during social interaction, and in Experiment 3, DZP increased locomotor activity in a Figure-8 maze. In Swiss mice, DZP (1 mg/kg) inhibited social interaction in the light–dark test [12] and in rats, a non-sedating dose of DZP decreased walking in intruder rats during an anticipation of confrontation [50]. The finding that mice that received 2.0 mg/kg displayed behavioral inhibition in their home cage during mildly threatening tests, but not during direct social interaction supports suggestions that DZP effects are context dependent, and demonstrates the utility of these three subtests, each with an increasing level of threat exposure.

Straub tail was reduced by acute DZP (2 mg/kg) during social interaction (Experiment 1). Straub tail can be induced by opioid and dopamine agonists [27] and is a component of the serotonin syndrome [45]. Straub tail was increased in SD mice (Experiment 2) and there was a nonsignificant trend for increased Straub tail in SD hamsters [28]. Swim stress-induced Straub tail in rats was reversed by a μ opioid antagonist [31]. The current findings that acute DZP decreased Straub tail agree with findings that DZP inhibited morphine-induced Straub tail [16]. The mechanisms of SD-induced Straub tail and the reversal by DZP remain to be determined.

We currently report effects of prior acute exposure to DZP. In Experiment 2, we hypothesized that DZP administered after SD would affect behavior 24 h later through inhibition of memory consolidation. Although a low dose of DZP (1 mg/kg) had amnesic effects in mice [41], the low doses of DZ used in the current study did not block acquisition of generalized fear. However, behavioral measurements 24 h following acute DZP exposure indicated effects of prior DZP exposure, regardless of defeat status; these effects may have been secondary to DZP withdrawal. Most studies that examine BZ withdrawal effects use chronic drug administration [22]. Withdrawal effects (increased rearing and hyperactivity) from a single dose of BZ have been observed in both mice and man, despite lack of receptor occupancy [20,55]. In rats, the half-life of DZP and its metabolite desmethyldiazepam are 0.88 and 1.11 h, respectively [23]; therefore, it is unlikely that in Experiment 2, DZP remained in the system 24 h after exposure. During habituation, mice that received 2.0 mg/kg DZP 24 h prior to the resident–intruder test displayed increased locomotor activity and more approaches and rearings relative to VEH. During the barrier subtest, mice, SD as well as NOSD, that received 2.0 mg/kg DZP 24 h prior had increased barrier avoidance and ear wiggling, behaviors typically displayed more by defeated mice (e.g., Experiment 2). Ear wiggling is a proceptive behavioral component of female rat sexual behavior (reviewed in Ref. [17]) that is increased by chronic psychosocial stress in female rats [56]. Ear wiggling in female rats is affected by the hormonal state of the stimulus rat [53]. To our knowledge, ear wiggling in socially stressed male mice has not been described previously. Whether the physiological state of the OBX stimulus mouse elicited ear wiggling in SD mice is unclear. During social interaction, there was a nonsignificant trend for SD mice that received 2.0 mg/kg DZP immediately after SD to have increased Straub tail 24 h later. Since DZP inhibited Straub tail in Experiment 1, and in morphine-treated mice [16], these findings may be secondary to withdrawal from DZP. Tail elevation and increased anxiety have both been observed following withdrawal from chronic DZP (reviewed in Ref. [20]).

In NOSD mice, some effects of prior DZP exposure were similar to defeat-induced behavioral changes or anxiogenic responses. NOSD mice treated with 2.0 mg/kg had more risk assessment (SAP) and crouch defense than VEH. In hamsters, DZP (6 mg/kg, ip) increased defensive posture 24 h later, but only in SD hamsters [28]. Acute administration of DZP decreased defensive posture in mice [35], but in another study, a BZ increased defensive posture in mice exposed to anosmic stimulus mice [18], as used in Experiment 2. We did not observe an effect of DZP on defensive posture in mice tested under the influence of DZP. Whether increased defensive posture and risk assessment 24 h after DZP exposure are related to DZP withdrawal is unclear.

The current experiments provide a comprehensive ethogram of social stress-induced behaviors in DBA/2mice, including avoidance, defense, risk assessment, Straub tail and ear wiggling. DZP induced different behavioral responses depending on the degree of the stress exposure, demonstrating that the expression of DZP-induced behavioral disinhibition varies depending on context. Low doses of DZP did not block SD-induced fear responses, but did induce possible withdrawal effects indicative of anxiety. Whether these effects of DZP on agonistic behaviors generalize to other mouse strains remains to be determined. These findings may relate to clinical anxiety disorders in which DZP is either ineffective or provokes further anxiety. Similarly, different degrees of threat exposure elicit a variety of animal behaviors that may be used to model diverse symptoms of clinical anxiety disorders and to screen pharmacological effects.

Acknowledgments

The authors thank Elaine M. Hull, Maurice Sipos and George A. Saviolakis for editorial comments, and Corenthian Booker, Christopher Robison, Wan Keung Chen and Ivonna Smith for assistance with behavioral scoring. Lucille A. Lumley PhD was supported as a National Research Council Fellow.

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