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The Effects of Ethanol and Diazepam on Reactions to Predatory Odors[1]

ROBERT J. BLANCHARD,*† D. CAROLINE BLANCHARD,†[2] SCOTT M. WEISS† AND SCOTT MEYER*

**Department of Psychology and †Bekesy Laboratory of Neurobiology, University of Hawaii*

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BLANCHARD, R. J., D. C. BLANCHARD, S. M. WEISS AND S. MEYER. *The effects of ethanol and diazepam on reactions to predatory odors*. PHARMACOL BIOCHEM BEHAV **35**(4) 775–780, 1990.—In a straight alley containing a cat odor stimulus rats show high rates of risk assessment, including flat back approach and stretch attend behaviors oriented toward the threat stimulus and contact with the stimulus. In this situation, diazepam (2.0 and 4.0 mg/kg) significantly reduced risk assessment measures (flat back approach + stretch attend), while not reliably altering control locomotion (curved back). In combination with earlier findings that the same doses of diazepam reliably increased risk assessment from a movement arrest baseline, these results strongly support a behavioral model of anxiety involving predictable nonmonotonic changes in risk assessment as a function of anxiety reduction. In comparison to diazepam, ethanol had less pronounced effects on the Cat Odor Test, as in earlier tasks of reactivity to potential threat. However, reliable dose × time interactions for risk assessment measures suggest ethanol effects similar to those of diazepam but most pronounced in initial stages in the test session.

Anxiety Diazepam Ethanol Risk assessment Odor Predator Defense

WE have devised an Anxiety/Defense Test Battery (A/DTB) to tap two particular components of the natural defensive repertory of laboratory rats. These two patterns, risk assessment and interference with ongoing nondefensive behaviors, have been implicated as the longer lasting components of defensive behavior to situations or stimuli associated with potential threat (3). These long durations, 24 hours or more in some cases, present problems for pharmacological studies. However, the A/DTB by using less intense stimuli, or partial stimuli, enables a compression of this time scale.

Risk assessment behaviors include orientation to and visual scanning of a potentially threatening stimulus, together with approaches and contact to that stimulus. They may occur in conspecific threat situations (9), to stimuli associated with shock (10,11) and to stimuli associated with a predator (3) as well as partial predator stimuli [cat odors, (4)]. Risk assessment behaviors can have both a long latency and a long duration, particularly when very intense threat stimuli are used. The long-latency risk assessment onset associated with intense threat reflects a defense sequence in which flight and distancing from the threat stimulus and immobility (crouching or freezing) typically occur over minutes or hours, with overt risk assessment appearing only as these initial reactions decline (4). Anxiety-reducing agents may thus produce a *biphasic* effect on risk assessment, depending on the types of defensive behaviors typical of control subjects at that point: when control levels of defense involve flight, immobility and distancing from the threat stimulus, then anxiolytics should increase risk assessment behaviors. However, when high levels of risk assessment are typical of control subjects, anxiolytics should decrease risk assessment and promote a return to normal, nondefensive behaviors.

The first two tasks comprising the Anxiety/Defense Test Battery, a Light-Dark Box and an Eat-Drink Box, both involved the possibility of brief exposure to a live cat separated from the subject by a screen. The reactions of cat-exposed subjects after such experience included high levels of freezing and movement arrest (2), indicating that if the model described above (3,4) is correct, then anxiolytic action should result in an increase in risk assessment. Consistent with this analysis, diazepam (2–4 mg/kg) increased 4 out of 5 measures of risk assessment in these tests. In contrast, 6 of the 7 measures of nondefensive behavior (e.g., eating, drinking, offense, sex) which might have been "released" by anxiety-reducing agents failed to show any effect of diazepam (2). Although ethanol (0.6–1.2 g/kg), another putative anxiolytic, produced fewer changes in the initial test session (5), these changes again appeared to be somewhat selective: one risk assessment behavior was increased while increases in another approached statistical significance. Other risk assessment measures increased on a retest day, but the interpretation of these changes is less clear. Since both of these tasks produced high levels of freezing in cat-exposed vehicle control subjects, the increases in risk assessment measures for both diazepam- and ethanol-treated subjects are congruent with the analysis of risk assessment as a particularly central measure of anxiety, with diazepam having the more potent effect. In the present studies, the same doses of diazepam and ethanol are used in the third task of

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[2]Requests for reprints should be addressed to D. Caroline Blanchard.

the Anxiety/Defense Test Battery. This Cat Odor Test uses the odor of a cat to elicit defensive behaviors in rat subjects. While predator odors do elicit defensive reactions in a variety of species including rats (4, 7, 12), this partial predator stimulus might be expected to elicit a less intense reaction than the actual presence of a live cat, such as was used in the Light-Dark Box and the Eat-Drink Box. Thus, in the cat odor test, from a baseline of risk assessment, ethanol, and especially diazepam, would be expected to reduce risk assessment behaviors.

EXPERIMENT 1: ETHANOL

METHOD

Subjects

The subjects were singly housed adult Long-Evans rats from breeding stock maintained by the University of Hawaii Laboratory Animal Services. Each group was composed of 10 male (average weight 420 g) and 10 female rats (average weight 292 g).

Ethanol Dose

Ethanol was given in 0.6 and 1.2 g/kg doses, in a physiological saline vehicle. All doses were injected IP 30 min prior to testing in a constant volume of 3 cc per kg body weight. The same volume of vehicle was used as the control.

Procedure

Cat Odor Test. The test was run in a box 120 cm long, 30 cm high and 15 cm in width. The front of the box was a clear Plexiglas sheet, and the back wall was marked at 10 cm intervals. The top of the box was constructed of wire mesh screening. A number of $9 \times 9 \times 2$ cm wood blocks wrapped in cloth served as the cat odor or control stimuli. To produce cat odor stimuli, the cloth used to cover the block was left overnight in the cage of a laboratory-housed domestic cat. Just prior to the test session this cloth was rubbed vigorously against the cat's fur for 3 min. The block was attached by an eye-hook to one end of the cat odor text box to prevent the subject moving it out of position.

Twenty min after injection of ethanol or vehicle control, the subject was placed near the center of the cat odor box and facing away from the stimulus block. The 10-min session was videorecorded and later analyzed to measure the frequency and durations of curved back approach (normal progression toward the stimulus); flat back approach to the stimulus (top of the back is lower than the ears when the ventral surface of the snout is held parallel to the ground); stretch attend (animal stands oriented toward the stimulus with back lower than the ears; from this position it may lean forward toward the stimulus, but locomotion in this posture is classified as flat back approach); contact with the stimulus.

Data Analysis

Results were analyzed by ANOVA for the three treatment groups. Newman-Keuls analyses were used to evaluate the significance of individual dose differences for measures showing significant ethanol dose effects. Because ethanol has previously been shown to selectively impact attack behavior in the initial stages of confrontation with a potentially threatening conspecific (6), data are presented in terms of the first and second 5-minute blocks of the test session.

RESULTS

Durations of curved back approach, flat back approach, stretch attend, and contact with the cat odor stimulus for vehicle and ethanol groups in the first and second 5-min blocks are presented in Fig. 1.

Stretch Attend/Flat Back Approach

The main effects of dose, $F(2,53) = 3.44$, $p<0.05$, sex, $F(1,53) = 6.50$, $p<0.02$, and time, $F(1,53) = 21.49$, on the frequency of stretch attend and flat back approach were statistically significant. Subsequent Newman-Keuls analysis indicated that the saline and the 1.2 g/kg groups were different, with the latter showing fewer stretch attend/flat back approaches. Females had a higher frequency of these behaviors than males (F mean = 3.45, M mean = 1.86), and more stretch attend/flat back approaches were seen in the first 5 min of the test than in the second and final 5-min period.

With the exception of sex effects, which approached, but failed to reach an acceptable level of statistical significance (F mean = 10.95 sec, M mean = 7.05 sec), the duration of stretch attend/flat back approach data presented a similar pattern. Dose effects, $F(2,53) = 3.54$, $p<0.05$, and time effects, $F(1,53) = 14.38$, $p<0.001$, were both reliable, and subsequent Newman-Keuls analysis again indicated that the 1.2 g/kg group and the saline control groups were again different. As with frequency, duration of stretch attend/flat back approach was reduced in the high dose ethanol group, and over time in the situation. None of the interactions of these factors was reliable for either frequency or duration.

Curved Back Approach

Ethanol effects on frequency of curved back approach were not reliable, $F(2,53) = 0.14$, $p>0.05$, nor were sex effects statistically significant, $F(1,53) = 1.55$, $p>0.05$, for this measure. Time (first vs. second 5 min of test) was significant, $F(1,53) = 28.35$, $p<0.0001$, with fewer instances of curved back approach seen during the second half of the 10-min test session. Results for duration of curved back approach provided a similar pattern, with drug and sex effects failing to achieve an acceptable level of statistical significance, while time effects were again reliable, $F(1,53) = 25.23$, $p<0.0001$.

Contact

Contact frequency showed no reliable effects of either dose or subject sex. Time effects were reliable, $F(1,53) = 54.51$, $p<0.0001$, as was the dose $\times$ time interaction, $F(2,53) = 6.65$, $p<0.005$: more contacts occurred during the first 5 min of the test period than during the last 5, particularly for the two ethanol groups. Subsequent analysis indicated that the early-late differences were reliable for each of the two ethanol groups 0.6 g/kg, $t(18) = 4.97$, and 1.2 g/kg, $t(18) = 5.70$, both $p<0.01$, but not for the saline control group.

None of these effects was reliable for contact duration.

EXPERIMENT 2: DIAZEPAM

METHOD

Subjects

The subjects were singly housed adult Long-Evans rats from breeding stock maintained by the University of Hawaii Laboratory Animal Services. Each group was composed of 6 male (average weight 454 g) and 6 female rats (average weight 280 g).

Diazepam Dose

Diazepam was given in 2 and 4 mg/kg doses, in a vehicle

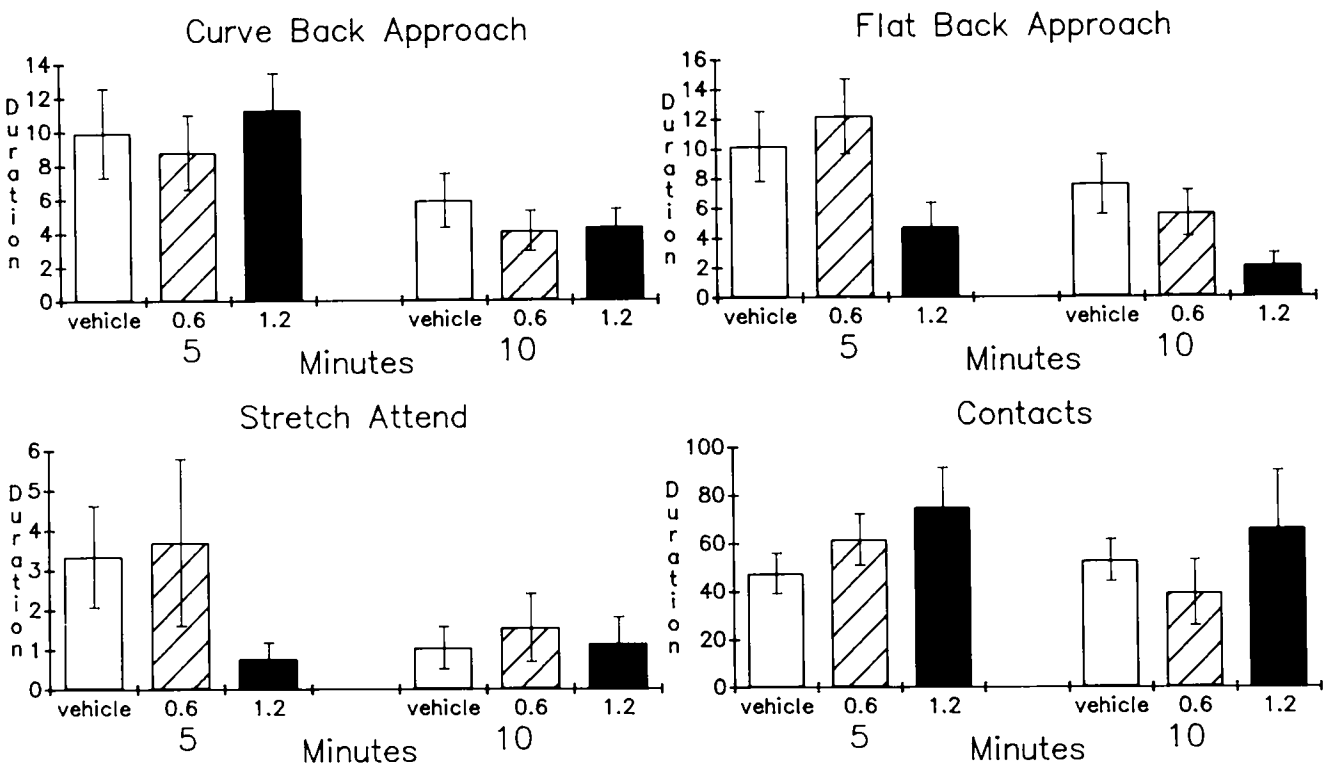

FIG. 1. Durations of curved back approach, flat back approach, stretch attend and contacts for vehicle, 0.6 and 1.2 g/kg ethanol groups during the first and second 5-min blocks of the Cat Odor Test.

consisting of 25% propylene glycol, 3% ethyl alcohol, and 72% double distilled water. All drugs were injected IP 30 min prior to testing in a constant volume of 3 cc per kg body weight. The same volume of vehicle was used as the control.

Procedure

The apparatus and procedure were identical to those of the previous study, except that a longer (20 min) test session was used. In addition to the measures taken in that study, a time sampling procedure was used to assess location of the subject within the cat odor alley, with locations noted from segment 1 (the end of the alley opposite the stimulus) through segment 9 (the segment containing the stimulus). In this time sampling procedure, the following behaviors were also noted: lying, crouching, standing, rearing, locomotion, and grooming. In order to determine if the time sampling procedure was able to detect the same changes as did the real-time analysis, curved back approach, flat back approach, and stretch attend were also rated.

RESULTS

Durations of curved back approach, flat back approach, stretch attend, and contacts for vehicle and diazepam groups in the cat odor test are presented in Fig. 2.

Stretch Attend and Flat Back Approach

Both dose, $F(2,29)=4.47$, $p<0.02$ and time, $F(1,29)=4.40$, $p<0.05$, effects on frequency of stretch attend and stretch approach were reliable. Subsequent Newman-Keuls analysis indicated that both diazepam doses were different than the control on both measures, showing less stretch attend and stretch approach than did the vehicle control group. Dose effects on duration were also reliable, $F(2,29)=5.17$, $p<0.02$. Subsequent Newman-Keuls analysis indicated that both 2.0 and 4.0 mg/kg doses showed reduced durations in comparison to the control level.

Curved Approach

Dose effects on curved back approach were not reliable for either frequency, $F(2,29)=0.63$, $p<0.05$, or duration, $F(2,29)=0.71$, $p<0.05$, measures. The effect of time within session was reliable for both frequency, $F(1,29)=17.34$, $p<0.0001$ and duration, $F(1,29)=10.89$, $p<0.005$, with a reduction in curved back approach in the second half of the session.

Contact

Dose effects were not reliable for either contact frequency, $F(2,29)=0.76$, $p>0.05$, or duration, $F(2,29)=0.79$, $p<0.05$. The effect of time within session was reliable for contact frequency, $F(1,29)=14.36$, $p<0.001$, with contact frequency declining in the second half of the test session.

Time Sampling Measures

Results of behavior measures for the time sampling procedure are presented in Table 1. Ratings of locomote (note that both curved and stretch approach would be included within this measure, as would locomotion away from the cat odor stimulus), $F(2,29)=5.91$, $p<0.01$, groom, $F(2,29)=4.93$, $p<0.02$, rear, $F(2,29)=5.45$, $p<0.001$ and crouch, $F(2,29)=10.79$, $p<0.001$,

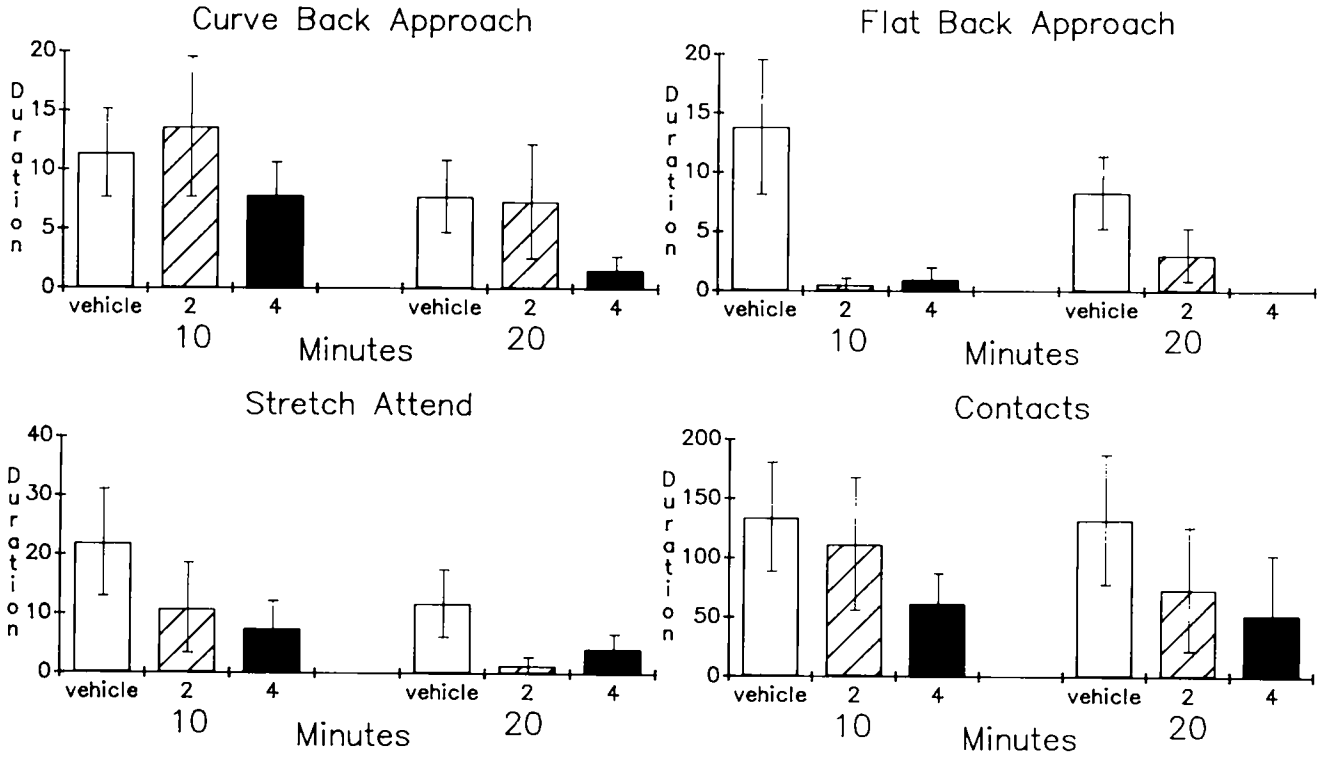

FIG. 2. Durations of curved back approach, flat back approach, stretch attend and contacts for vehicle, 2.0 and 4.0 mg/kg diazepam groups during the first and second 10-min blocks of the Cat Odor Test.

were each reliably different as a function of dose level, with the first 3 measures declining, and the last increasing, with increasing diazepam doses. Subsequent Newman-Keuls analyses indicated that in each case (except grooming, where only the 4.0 g/kg group was different from the vehicle group) both diazepam doses were different from the control level.

Diazepam dose effects on location within the cat odor alley were not reliable for any of the 9 segments. Sex effects were also not reliable for any specific segment. However, it is notable that females were about twice as likely as males to be located within the cat odor stimulus segment.

DISCUSSION

In contrast to the other two tests of the Anxiety/Defense Test Battery risk assessment, behaviors (stretch attend, flat back approach, and contact with the cat odor stimulus) were common during the initial time period of this test for both control and drug-treated subjects. In this respect the present results are similar to those obtained by Pinel and Mana (10) using a prod through which subjects had received a single shock: they obtained flat back approach durations of 35% of total time when a relatively severe 4 mA shock was used. The control subjects for the two present experiments averaged about 25% risk assessment (flat back approach, stretch attend, and contact) durations during the test period. This is an important consideration, as the expected effect on risk assessment of anxiolytic compounds depends on the control level of risk assessment, with increased risk assessment accompanying reduced anxiety when movement arrest is the predominant control reaction, and decreased risk assessment seen with reduced anxiety against a background of high risk assessment. This complex relationship occurs because the latency of risk assessment reflects the intensity of threat, with high levels of threat producing a long latency to approach and explore the threat situation or stimulus (4), with the latency decreasing as the intensity of threat decreases.

The results of the present tests are completely in agreement with this analysis, in that the present partial cat stimulus (cat odor) elicited higher levels of risk assessment than did a situation in which a live cat was presented (2,5): compare the present 24% crouching ratings to approximately 80% crouching ratings obtained in those studies. Moreover, in contrast to the consistent pattern of *increased* risk assessment for diazepam-treated subjects in the other tests of the Anxiety/Defense Test Battery, diazepam consistently and reliably *reduced* risk assessment in the present cat odor test. Curved back approach, in contrast, did not change as a function of diazepam dose, suggesting that activity differences

TABLE 1

PERCENT RATINGS OF VARIOUS BEHAVIORS FOR VEHICLE CONTROL, 2.0 AND 4.0 mg/kg DIAZEPAM GROUPS IN A CAT ODOR TEST

	Vehicle	2.0	4.0
Lie	4.01	1.44	3.55
Crouch	23.78	52.49*	73.11*
Rear	20.83	8.32*	7.50*
Stand	6.54	5.28	1.88
Locomote	26.74	16.05*	9.80*
Groom	18.10	16.42	4.17*

*Indicates measures that are significantly different from vehicle ($p<0.05$ or less).

were not an important factor in the stretch attend/flat back approach effects of diazepam. The lack of reliable curved back approach effects for diazepam does not completely eliminate the possibility that sedative effects may have contributed to the present findings, as some other active behaviors (rear and groom) were also reduced by diazepam, a finding which partially replicates earlier A/DTB results (2). Although the question of sedative effects for these doses requires further examination, the pattern obtained does suggest that those activities most clearly associated with risk assessment were especially sensitive to diazepam.

The diazepam results of this study are thus consistent with the diazepam findings of the early Anxiety/Defense Test Battery tests, but given that the diazepam effects are opposite in sign in the two situations, only when interpreted in the light of a nonmonotonic relationship between the intensity of anxiety/threat and the magnitude of risk assessment.

The ethanol results for the cat odor test were similar to, though somewhat weaker than, those of diazepam in that reliable reductions in stretch attend/flat back approach were obtained. While the ethanol contact effects were not reliable overall, the significant dose × time interaction reflects a finding that both ethanol groups made more contacts with the cat odor stimulus in the first half of the test session, and fewer in the second half, while control contacts were about even for early and late periods in the test. As with the diazepam comparisons, curved back approaches were not different for the ethanol as opposed to control groups, suggesting a lack of important sedative effects for subjects at these ethanol dose levels.

These results, suggesting some significant anxiolytic action of ethanol on risk assessment behaviors, but a pattern of effects which was less clear and consistent for ethanol than for diazepam, is very similar to the pattern obtained for the other tests of the A/DTB (2,5). The present ethanol study did not include a time-sampling component, and the only nonrisk assessment behavior measured here was curved back approach. Thus, although the curved back results showed no reliable ethanol effects, the present study cannot clearly differentiate risk assessment effects of ethanol from ethanol effects on a range of nonrisk assessment behaviors. It does, however, provide considerable support for a view that ethanol has anxiolytic effects which are particularly visible in the initial moments in a threatening situation (6).

Time Sampling Measures

The time sampling measures of the present study were taken, in part, to establish the movement arrest (crouching) baseline against which changes in risk assessment could be evaluated more meaningfully. However, these measures also included grooming, which is of interest because it increased with higher diazepam doses in the other tests of the A/DTB (2), a diazepam effect which was obtained here as well. Since grooming, regarded as a nondefensive behavior, should decrease with higher levels of defensiveness and increase following anxiolytic drug action, this consistent finding is puzzling. What it may reflect is a direct action of diazepam, possibly on sensory mechanisms, as diazepam appears to decrease grooming in a variety of situations (8).

While the directly measured durations of curved back approach did not change with diazepam treatment, it is notable that the active behaviors measured in the time sampling procedure, locomote and rear, did tend to decline reliably, while crouching increased reliably. This last finding is, on first examination, puzzling, as crouching is typically taken to reflect movement arrest. However, the distinction between crouching, an active immobile posture involving high levels of muscle tension, and sitting, posturally very similar but involving little muscle tension, is very difficult to make from videotape records. It appears very likely that the increased "crouching" obtained for the diazepam groups in the present study actually reflected a shift from greater to lesser activity with diazepam. This view, consistent with decreases in active behaviors for the time sampling procedure, may suggest some sedative effects of diazepam at these doses which was not indicated by the curved back measure discussed above. Certainly this pattern of results provides an incentive for further attempts to clarify the relationship between possible anxiolytic and sedative effects of diazepam as well as other potential anxiety-impacting drugs.

Sex Differences

Because the earlier portions of the A/DTB (2,5) suggested a pattern of sex differences in anxiety, sex differences in the present tests are particularly interesting. In those earlier studies, sex effects could be meaningfully analyzed in terms of comparisons involving behavioral controls, i.e., comparisons between cat-exposed and nonexposed vehicle controls: females tended to show more of those behaviors which increased with cat exposure, and less of those which declined for the cat-exposed group, strongly suggesting greater anxiety. In the present procedures there were no behavioral (as opposed to drug) controls, so these comparisons were not possible. However, in the present ethanol study, sex had reliable effects on frequency of stretch attend/flat back approach and the sex effect on duration of these behaviors approached, and just failed to reach, an acceptable level of statistical significance. In both cases, females showed more of the behavior, suggesting greater anxiety. No significant main effects of sex were found in the diazepam study, but it is notable that the females in that study did spend about twice as much time in close proximity to the cat odor stimulus (Location 9 in the cat odor box) than males which, in this context (of anxiolytics reducing risk assessment), again suggests that females were more anxious than males. These results are in agreement with an emerging body of data suggesting that in rats, as in humans (1), females may display more behavioral anxiety than males. Thus, both the sex difference data and the drug dose effects in the cat odor test indicate that the Anxiety/Defense Test Battery may provide a much more precise animal model than has previously been available for analysis of anxiety and its relationship to independent variable effects.

REFERENCES

1. Barlow, D. H. Anxiety and its disorders: The nature and treatment of anxiety and panic. New York: Guilford Press; 1988.
2. Blanchard, D. C.; Blanchard, R. J.; Tom, P.; Rodgers, R. J. Diazepam reduces risk assessment in an Anxiety/Defense Test Battery. Psychopharmacology (Berlin); in press.
3. Blanchard, R. J.; Blanchard, D. C. Anti-predator defensive behaviors in a visible burrow system. J. Comp. Psychol. 103:70–82; 1989.
4. Blanchard, R. J.; Blanchard, D. C.; Hori, K. Ethoexperimental approaches to the study of defensive behavior. In: Blanchard, R. J.; Brain, P. F.; Blanchard, D. C., eds. Ethoexperimental approaches to the study of behavior. Dordrecht: Kluwer Academic Publishers; 1989: 114–136.
5. Blanchard, R. J.; Blanchard, D. C.; Weiss, S. M. Ethanol effects in an Anxiety/Defense Test Battery. Submitted.
6. Blanchard, R. J.; Flannelly, K. J.; Hori, K.; Blanchard, D. C.; Hall, J. Ethanol effects on female aggression vary with opponent size and time within session. Pharmacol. Biochem. Behav. 27:645–648; 1987.
7. Cattarelli, M.; Vigouroux, M. Evaluation des reactions emotionelles declenchees par des odeurs a signification biologique chez le rat au course d'un conditionnement operant. Physiol. Behav. 71:223; 1981.

8. File, S. E.; Mabutt, P. S.; Walker, J. H. Comparison of adaptive responses in familiar and novel environments—modulatory factors. Ann. NY Acad. Sci. 525:69–79; 1988.
9. Grant, E. C.; Mackintosh, J. H. A comparison of the social postures of some common laboratory rodents. Behaviour 21:2146–259; 1963.
10. Pinel, J. P. J.; Mana, M. J. Adaptive interactions of rats with dangerous inanimate objects: Support for a cognitive theory of defensive behavior. In: Blanchard, R. J.; Brain, P. F.; Blanchard, D. C., eds. Ethoexperimental approaches to the study of behavior. Dordrecht: Kluwer Academic Publishers; 1989: 137–150.
11. Van der Poel, A. M. A note on "stretched attention," a behavioral element indicative of an approach-avoidance conflict in rats. Anim. Behav. 27:446–450; 1989.
12. Vernet-Maury, E. Trimethyl-thiazoline in fox feces: A natural alarming substance for the rat. In: Van der Starre, H., ed. Olfaction and taste VII. Washington, DC: IRL Press, Ltd.; 1980.