*Behavioural Pharmacology (1995)*, **6**, 732–745

# A model of 'antipredator' defense in Swiss-Webster mice: effects of benzodiazepine receptor ligands with different intrinsic activities

G. Griebel,[1] D.C. Blanchard,[1,2] A. Jung[3] and R.J. Blanchard[1,3]

[1] *Békésy Laboratory of Neurobiology;* [2] *Department of Anatomy and Reproductive Biology, John A. Burns School of Medicine; and* [3] *Department of Psychology, University of Hawaii, 1993 East-West Road, Honolulu, HI 96822, USA*

*Correspondence to: G. Griebel, Synthélabo Recherche (L.E.R.S.), 31 ave. P.V. Couturier, 92220 Bagneux, France.*

**A mouse defense test battery (MDTB) has been designed to assess defensive reactions of Swiss-Webster mice to situations associated with nonpainful threat. When compared to mice approached by a leather glove, animals confronted with an anesthetized or a conscious rat displayed potentiated flight responses and defensive threat/attack reactions, while risk assessment performances were generally similar in all three conditions. Furthermore, escape attempt responses following removal of the stimulus were higher in the conscious rat condition compared to the two other groups. Taken together, these results suggest that flight reactions and defensive threat/attack responses are specific to the rat, and thus indicate that the MDTB may relate to 'antipredator' defense. In mice confronted with an anaesthetized rat, administration of the benzodiazepine (BZ) receptor full agonist chlordiazepoxide (5–25 mg/kg, i.p., 30 min) and the BZ partial agonist Ro 19-8022 (0.5–2 mg/kg, i.p., 30 min) altered one of two risk assessment measures and inhibited defensive attack behaviors, but failed to counter the post-predator increase in escape attempts. In addition, Ro 19-8022 also strongly reduced flight responses. The overall behavioral profile suggests a fear/anxiety-reducing action of both drugs. By contrast, administration of the BZ inverse agonist Ro 19-4603 (0.025–0.1 mg/kg, i.p., 30 min) reliably released these defensive responses. Interestingly, the BZ antagonist flumazenil (5–20 mg/kg, i.p., 30 min) manifested differential intrinsic activity depending upon the level of threat. Thus, in a weakly threatening situation, the drug potentiated flight reactions, indicating an inverse agonist-like action, decreased defensive biting in a highly threatening situation, indicating an agonist activity. These findings demonstrated that BZ ligands differently modulated 'antipredator' defense in Swiss-Webster mice, depending upon their intrinsic (positive or negative) efficacy, but also depending upon the defense strategy required by the threat.**

**Keywords: 'Antipredator' defence – Anxiety – Benzodiazepine receptor ligands – Chlordiazepoxide – Flight – Flumazenil – Risk assessment – Ro 19-4603 – Ro 19-8022 – Swiss Webster mouse**

## INTRODUCTION

Benzodiazepine (BZ) receptors bind a vast number of compounds with differential efficacy on a continuum from full positive to full negative intrinsic efficacy (Braestrup *et al.*, 1984; Haefely, 1989). Thus, BZ receptor agonists such as the conventially used anxiolytics and anticonvulsants (e.g. chlordiazepoxide, diazepam, alprazolam) have a high capacity to activate the receptor (positively) and are considered as BZ receptor full agonists. Recently the concept of BZ agonist has been elaborated to encompass a class of compounds with lower intrinsic efficacy, described as partial agonists (e.g. pipequaline, bretazenil). Clinical studies have shown that these ligands retain anxiolytic activity, but are devoid of marked sedation (Willer *et al.*, 1986; Katschnig *et al.*, 1991). Beside these categories, there are two other classes of ligand that also bind to the BZ receptor. One class includes antagonists such as flumazenil and ZK 93426, which show high affinity at the receptor but have low to absent intrinsic activity (Haefely, 1983; Jensen, 1984). The other is a class of ligands called inverse agonists (e.g. $\beta$-CCM, Ro 15-3505), which have actions which are exactly opposite to those of the agonists and thus are anxiogenic in humans (Dorow *et al.*, 1983; Gentil *et al.*, 1990).

A large preclinical literature indicates that BZ full and partial agonists almost invariably elicit anxiolytic-like responses, while inverse agonists often induce an opposite effect (for a review, see Treit, 1991). Furthermore, studies with flumazenil either showed a lack of effect or indicated that the drug increased anxiety-like responses in animals

(File *et al.*, 1982; Treit, 1991). Studies directly measuring components of the monkey and rodent defensive repertory have revealed that BZ full agonists, at nonsedative doses, tend to reduce some but not all defensive reactions to a variety of threat stimuli (for review, see Rodgers, 1991; Blanchard *et al.*, 1994). Some of these effects appear to be selectively characteristic of drugs with efficacy against clinical anxiety (Blanchard *et al.*, 1993b). Furthermore, as might be predicted, BZ inverse agonists (e.g. $\beta$-CCM, FG7142) have been found to stimulate defensiveness, while flumazenil was devoid of intrinsic activity (see Rodgers, 1991), although one report of increased defensive reactivity in timid mice has been published (Poshivalov, 1987). In a series of tests designed to elicit the full range of 'antipredator' defensive responses in wild and laboratory rats (the Fear/Defense Test Battery (F/DTB) and the Anxiety/Defense Test Battery (A/DTB)), we have recently observed that the BZ full agonists chlordiazepoxide, diazepam and midazolam markedly reduced defensive threat and attack behaviors, while effects on freezing and flight were more variable, differing substantially as a function of stimulus context (Blanchard *et al.*, 1989). Furthermore, chlordiazepoxide and diazepam reduced both proxemic avoidance, measured as location far from the predator or predator area, and also the threat-induced inhibition of eating and/or drinking. Finally, both compounds were found to have a consistent effect on risk assessment: increasing the response when the predator stimulus is strong and the baseline level low, inhibiting it when the stimulus is weak or partial, and risk assessment already predominant (Blanchard and Blanchard, 1990; Blanchard *et al.*, 1990).

These results suggest that when a wide variety of 'antipredator' defensive behaviors are measured, differential patterns of drug response may be associated with anxiolytic or anxiogenic action. The objective of the present study was to investigate further the validity of defense reactions as an index of anxiety, by assessing the behavioral effects in a test battery designed for use with mice of BZ receptor ligands displaying different intrinsic activities. Thus, we examined the effects of the BZ full agonist chlordiazepoxide, two recently developed and very potent BZ ligands, Ro 19-8022, a non BZ partial agonist (Facklam *et al.*, 1992; Jenck *et al.*, 1992) and Ro 19-4603, described as an inverse agonist (Pieri, 1988), and the BZ receptor agonist flumazenil.

This test battery was run in a (mouse-scaled) oval runway, based on that used in the rat F/DTB. However, specific situational and behavioral components of the A/DTB, involving reactivity to stimuli associated with potential threat rather than to the actual presence of an approaching predator (Contextual Defense Test), were incorporated into the mouse battery (Griebel *et al.*, 1995a,b). Furthermore, in order to demonstrate that the model may be considered as one relating to 'antipredator' defense, a first experiment was designed to compare the impact of different stimuli (leather glove, anesthetized rat, conscious rat) on defense responses of Swiss-Webster mice exposed to the runway paradigm.

## METHODS

### Animals

Subjects were 299 naive male Swiss-Webster mice obtained from Simonsen Laboratories (CA), 60–75 days old at the beginning of the experiment, and six Long-Evans male rats (400–500 g) bred in the laboratory. They were housed singly in polycarbonate cages in a room maintained under a 12 h light/dark cycle with light onset at 06.00 h.

### Apparatus

The test was conducted in an oval runway, 0.4 m wide, 0.3 m high, and 6.0 m in total length, consisting of two 2 m straight segments joined by two 0.4 m curved segments and separated by a median wall ($2.0 \times 0.30 \times 0.06$ m). The apparatus was elevated to a height of 80 cm from the floor, to enable the experimenter to hold the rat easily, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with videocameras mounted above the apparatus. Behavioral assessments were made from the recordings, with the observer unaware of the original pretreatment. Experiments were performed under red light between 13.00 and 17.00 h. After removal of each animal, the runway field was carefully mopped using hot soapy water to remove any residual odor due to urine, faeces or to the predator.

### Procedure

*Experiment 1: Effects of various stimuli on defense behavior in the oval runway.* Mice were randomly assigned to the following four groups: 'No stimulus', 'Leather glove stimulus', 'Anesthetized rat stimulus' and 'Conscious rat stimulus' ($n = 15$). In the 'No stimulus' condition, the pretest was followed by a 10 min period without predator exposure. After this period, the 3 min post-test was given. In the 'Leather glove' group, the experimenter wore a beige leather glove. Finally, in the 'Anesthetized rat stimulus' condition, stimulus animals were deeply anesthetized with 40 ml/kg of pentobarbital.

*Experiment 2: Effects of BZPR ligands on defense behavior in the oval runway.* Chlordiazepoxide HCl (RBI, Natick, MA), Ro 19-8022, flumazenil and Ro 19-4603 (F. Hoffmann-La Roche Ltd, Basel, Switzerland) were suspended in an isotonic saline vehicle to various concentrations, such that injections were always at a constant volume of 10.0 ml/kg. Mice were randomly assigned to the following four experiments: (a) chlordiazepoxide: control group ($n = 15$) and drug treatment groups (5 and 10 mg/kg, $n = 15$; 25 mg/kg, $n = 14$); (b) Ro 19-8022: control group and drug treatment groups (0.5, 1 and 2 mg/kg,: $n = 15$); (c) flumazenil: control group and drug treatment groups (5, 10 and 20 mg/kg, $n = 15$); (d) Ro 19-4603: control group and drug treatment groups (0.025, 0.05 and 0.1 mg/kg: $n = 15$). Mice received a single injection of either saline, chlordiazepoxide, Ro 19-8022, flumazenil or Ro 19-4603. Drugs were administered i.p. 30 min before the experiment was carried out. Mice were tested in an order randomized for drug treatment.

### The test battery

*Contextual defense.* Subjects were placed into the runway for a 3 min familiarization period, in which line crossings, wall rears, wall climbs and jump escapes were recorded [min 1 to 3]. The same behavioral parameters were also recorded during an equivalent period following tests involving exposure to the stimulus (post-test) [min 12 to 14]. Changes in the latter three (escape) measures during the post-stimulus period provide an index of contextual defense.

*Predator/stimulus avoidance test [min 4 to 6].* Immediately after the 3 min familiarization period, the stimulus rat or the leather glove was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. The experimenter stood adjacent to the runway while holding the anesthetized rat or wearing the leather glove. Approach was terminated when contact with the subject was made or the subject ran away from the approaching stimulus. If the subject fled, avoidance distance (the distance from the stimulus to the subject at the point of flight) was recorded. This was repeated five times.

*Chase/flight test [min 7 to 8].* The hand-held rat or the leather glove was brought up to the subject at a speed of approximately 2.0 m/s. The time it took to chase the subject a distance of 15 m was recorded. Overall flight speed (m/s) and maximum flight speed (measured when the subject is running straight over a 1 m segment) were subsequently calculated from these measures. In addition, the following parameters were recorded: number of stops (pause in movement) and orientations (subject stops, then orients the head toward the stimulus).

*Straight alley [min 9 to 11].* The runway was then converted to a straight alley by closing doors at both ends. Three approaches were made by the hand-held rat or the leather glove toward the subject in this inescapable runway, with pauses of 15 s each at 1.2, 0.8 and 0.4 m. Measures taken included immobility time, closest distance between the subject and the stimulus, and the number of approaches/withdrawals (subject must move more than 0.2 m forward from the closed door, then return to it). Finally, the experimenter brought the rat or the leather glove up to contact the subject. For each such contact, bites, vocalizations, upright postures and jump attacks by the subjects were noted. The contact procedure was repeated three times.

*Ledge test [min 15].* Subjects were placed on the median wall of the runway and the number of animals falling from the ledge in 30 s was scored as an indication of potential myorelaxant activity (this test was only performed in experiment 2).

### Statistics

Data from the chase/flight and the straight alley tests were analyzed by a single factor analysis of variance (ANOVA) or, for some infrequently occurring or highly variable behaviors, with the nonparametric Kruskal-Wallis ANOVA. Subsequent comparisons between treatment groups and control were carried out using Newman-Keuls procedures or the nonparametric Mann-Whitney U-test, respectively. In the contextual defense, rat avoidance/stimulus and forced contact tests, data were assessed by single or two-factor (drug × trial) ANOVA followed by either Newman-Keuls post-hoc comparison, Mann-Whitney U-test, or Wilcoxon matched pair procedure. Since the effects of trial and the drug × trial interactions in predator/stimulus and forced contact tests never reached a significant level, F values were not presented in these sections. Data from the ledge test were analysed by a $\chi^2$ procedure. Non parametric data are displayed as mean ± standard error in order to illustrate the group variation.

## RESULTS

### Experiment 1: Effects of various stimuli on defensive behavior in the oval runway

*Contextual defense: Motor activity before and after exposure to the stimulus (Fig. 1).* ANOVA revealed reliable main effects of stimulus for all behavioral mea-

sures [Line crossing: $F(3,56) = 3.17$, $p < 0.05$; wall rearing: $F(3,56) = 9.84$, $p < 0.001$ and escape attempt: $H = 29.29$, $p < 0.001$] and significant increases in line crossings ($p < 0.02$) and escape attempts (Wilcoxon matched pair test: $p < 0.001$) in the post-test period, relative to the initial 3 min pre-test; wall rearing [$F(1,56) = 1.02$] responses did not change in the post-stimulus period.

There were also significant stimulus type × test interactions for line crossing ($F(3,56) = 4.6$, $p < 0.01$) and escape attempts [Friedman: $N(1,60) = 37.23$, $p < 0.001$] but not for wall rearing [$F(3,56) = 2.62$]. Subsequent Newman-Keuls analyses indicated that line crossings significantly decreased in the post-test period in the NS group. Wilcoxon matched pair tests revealed significantly more escape attempts in the post-test period in LG, AR and CR conditions, but not in non exposed mice; escape attempts were also significantly higher in CR than in LG mice.

*Stimulus avoidance test (Fig. 2).* Kruskal-Wallis ANOVA indicated a significant stimulus effect on avoidance frequency [$H = 11.12$, $p < 0.005$] and the stimulus-subject distance at which avoidance occurred [$F(2,39) = 6.19$, $p < 0.005$]. Subsequent post-hoc analysis indicated that, when compared with LG condition performance, rat exposure markedly increased avoidance distance ($p < 0.005$ vs AR and $p < 0.001$ vs CR], and that mice from the CR group displayed significantly more avoidance responses than animals exposed to a leather glove or to an anaesthetized rat, [$p < 0.002$ vs LG and $p < 0.01$ vs AR].

*Flight/stimulus orientation test (Table I).* Kruskal-Wallis ANOVA failed to reveal significant stimulus effects on arrests in movement [$H = 3.48$] and orientation to

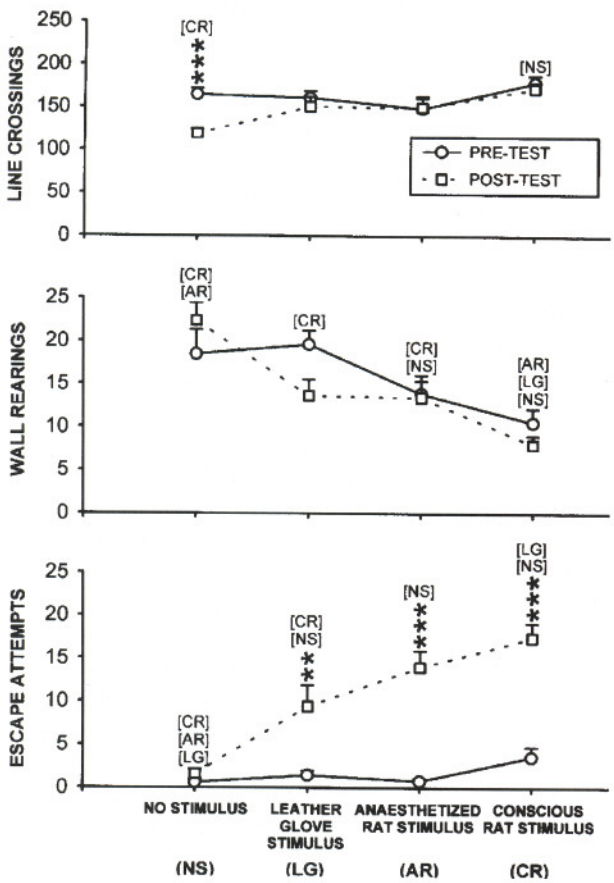


FIG. 1. Comparison of the impact of various stimuli on the frequency of three response measures before (pre-test) and after (post-test) exposure to the stimulus. Data points and vertical bars represent means and S.E.M. [NS], [LG], [AR] and [CR] indicate an effect that is significantly different [$p < 0.05$] from 'No Stimulus', 'Leather Glove Stimulus', 'Anesthetized Rat Stimulus' and 'Conscious Rat Stimulus' groups, respectively. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs pretest. In the 'No Stimulus' condition, the pretest was followed by a 10 min period without stimulus exposure, followed by the 3 min post-test.

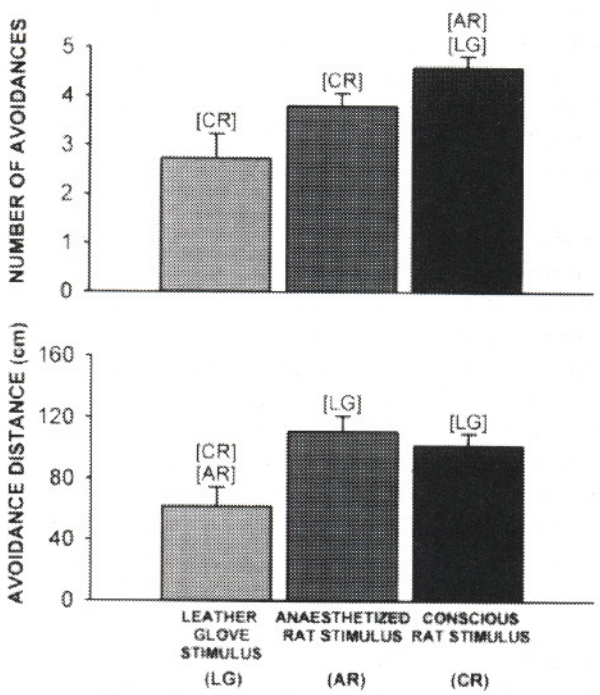


FIG. 2. Runway measures of avoidance for mice approached by various stimuli. Columns and vertical bars represent means and S.E.M. [LG], [AR] and [CR] indicate an effect that is significantly different [$p < 0.05$] from 'Leather Glove Stimulus', 'Anesthetized Rat Stimulus' and 'Conscious Rat Stimulus' groups, respectively.

TABLE I. Impact of various chasing stimuli on two speed measures and on the frequencies of stops and orientations toward the stimulus[1]

| | Overall speed (m/s) | Maximum speed (m/s) | Frequency of stops | Frequency of orientations |
|---|---|---|---|---|
| Leather glove | 0.37 ± 0.06†‡ | 0.67 ± 0.04†‡ | 11.27 ± 1.17 | 3.20 ± 0.74 |
| Anesthetized rat | 0.57 ± 0.04* | 1.09 ± 0.06* | 8.67 ± 0.83 | 4.93 ± 0.69 |
| Conscious rat | 0.69 ± 0.07* | 0.97 ± 0.04* | 8.47 ± 1.07 | 5.00 ± 0.75 |

[1]Data are presented as means ± S.E.M. *, † and ‡ indicate an effect that is significantly different [$p < 0.05$] from 'Leather Glove Stimulus', 'Anesthetized Rat Stimulus' and 'Conscious Rat Stimulus' groups, respectively.

Table II. Impact of various stimuli which remain at constant distance from the subject on the behavior of mice in the straight alley test[1]

| | Frequency of approaches/withdrawals | Closest distance between animals (cm) | Immobility time (s) |
|---|---|---|---|
| Leather glove | 3.47 ± 0.52 | 96.23 ± 16.32 | 3.21 ± 0.95†‡ |
| Anesthetized rat | 2.67 ± 0.46 | 122.67 ± 12.75 | 8.45 ± 2.32* |
| Conscious rat | 4.07 ± 0.48 | 94.33 ± 13.31 | 10.13 ± 1.71* |

[1]Data are presented as means ± S.E.M. *, † and ‡ indicate an effect that is significantly different [$p < 0.05$] from 'Leather Glove Stimulus', 'Anesthetized Rat Stimulus' and 'Conscious Rat Stimulus' groups, respectively.

the stimulus [$H = 3.84$], but there were significant main effects on overall [$F(2,42) = 8.2$, $p < 0.001$] and maximum [$F(3,56) = 19.61$, $p < 0.001$] flight speeds. Newman-Keuls post-hoc comparison showed that, when compared with LG subjects, mice from both rat stimulus groups ran faster from the chasing animal (Overall flight speed: $p < 0.02$ vs AR and $p < 0.001$ vs CR; maximum flight speed: $p < 0.001$ vs AR and CR).

*Stimulus approach: Straight alley test (Table II).* ANOVA revealed a significant overall effect of the stimulus on immobility time [$F(2,42) = 4.26$, $p < 0.02$], but not on the two other measures [frequency of approaches/withdrawals: $H = 3.6$; closest distance between animals: $F(2,42) = 1.24$]. Post-hoc analyses showed that immobility time was reliably higher in subjects exposed to a rat stimulus when compared to performances of mice approached by a leather glove.

*Forced contact with the stimulus (Fig. 3).* ANOVA indicated a significant effect of stimulus on frequency of biting the stimulus [$H = 29.65$, $p < 0.001$], jump attacks toward the stimulus [$H = 17.33$, $p < 0.002$] and vocalizations [$H = 21.29$, $p < 0.001$), but not for the frequency of upright postures [$H = 4.82$]. Subsequent Mann-Whitney U-tests revealed significant increases [$p < 0.002$] in biting, jump attacks and vocalizations in both rat stimulus groups compared to the LG condition. In addition, post-hoc analysis indicated reliably more biting of the conscious compared with the anesthetized rat [$p < 0.05$].

## Experiment 2: Effects of BZ ligands on defensive behavior in the oval runway

*Contextual defense: Motor activity before and after exposure to the predator (Fig. 4).* Chlordiazepoxide: comparisons (ANOVA) of chlordiazepoxide treated and saline control groups indicated significant effects of drug treatment on frequency of line crossings [$F(3,55) = 4.09$, $p < 0.01$] and wall rearings [$F(3,55) = 9.2$, $p < 0.001$), but not for the frequency of escape attempts [$H = 8.89$]. Newman-Keuls comparisons indicated a reliable increase in the number of line crossings at 10 mg/kg [$p < 0.05$ vs control] and a marked decrease in the frequency of wall rearings at 25 mg/kg [$p < 0.001$ vs control]. All behavioral measures increased significantly in the post-test period following presentation and removal of the predator [line crossings: $F(1,55) = 6.25$, $p < 0.02$; wall rearing: $F(1,55) = 6.1$, $p < 0.02$; escape attempts: Wilcoxon pair test: $p < 0.001$]. For line crossings there was a significant dose × test interaction [$F(3,55) = 3.29$, $p < 0.05$], which subsequent Newman-Keuls analysis showed to be due to a significant post-test increase in the group receiving the highest dose (25 mg/kg) of chlordiazepoxide [$p < 0.002$

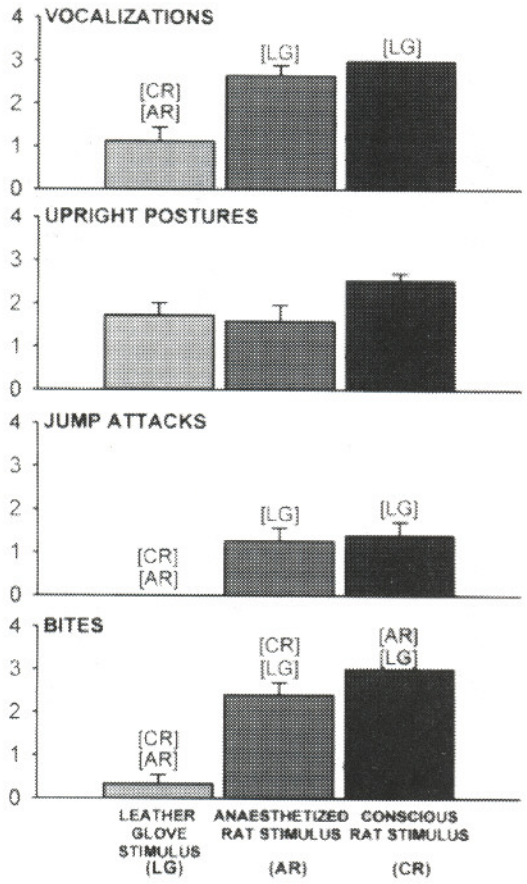


FIG. 3. Mean frequency of biting, defensive threat vocalization, upright posture and jump attacks to forced contact with a leather glove, a deeply anesthetized rat or a conscious rat. Columns and vertical bars represent means and S.E.M. [LG], [AR] and [CR] indicate an effect that is significantly different [$p < 0.05$] from 'Leather Glove Stimulus', 'Anesthetized Rat Stimulus' and 'Conscious Rat Stimulus' groups, respectively. There is no variability for the CR group on vocalizations and bites as we recorded three responses/three trials for each subject.

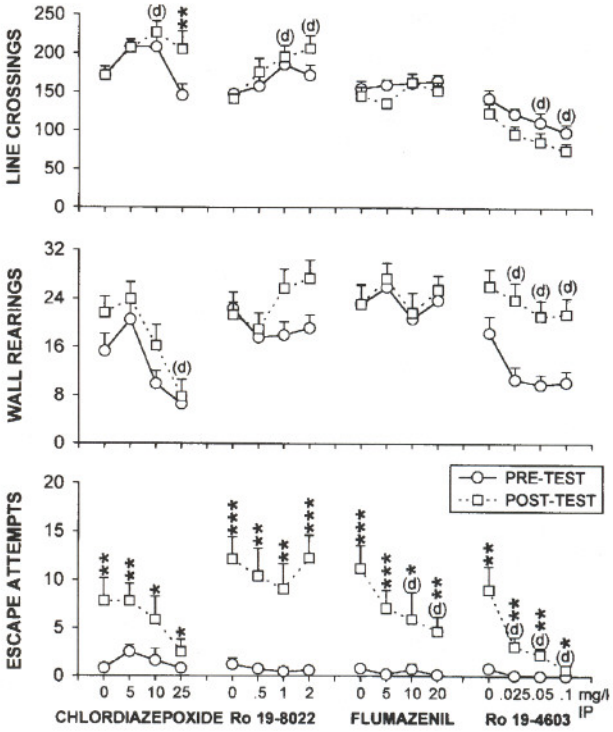


FIG. 4. Effects of a single acute dose of chlordiazepoxide, Ro 19-8022, flumazenil and Ro 19-4603 on the frequency of three response measures before (pre-test) and after (post-test) exposure to the predator. Data points and vertical bars represent means and S.E.M. (d) represents drug doses that are significantly different from vehicle [$p < 0.05$]. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.0001$ vs pretest.

versus pretest]. This interaction was not reliable for wall rearing [$F(3,55) = 0.49$]. Friedman ANOVA indicated reliable effects on escape attempts [$N(1,59) = 25.13$, $p < 0.001$] and subsequent analyses (Wilcoxon pair test) showed a post-test increase in this measure for both vehicle control and drug-treated groups.

Ro 19-8022: comparisons of Ro 19-8022 treated and saline control groups indicated that the drug had a significant overall effect on line crossing [$F(3,56) = 3.68$, $p < 0.02$], but not on wall rearing [$F(3,56) = 1.54$] or escape attempts [$H = 3.27$]. Newman-Keuls comparisons indicated a reliable increase in the number of line crossings at 1 and 2 mg/kg [$p < 0.05$ versus control for both comparisons]. All behavioral measures increased significantly in the post-test period following presentation and removal of the predator [line crossing: $F(1,56) = 5.19$, $p < 0.05$; wall rearing: $F(1,56) = 4.9$, $p < 0.05$; and escape attempts: Wilcoxon pair test on pre-post exposure means of all groups combined: $p < 0.001$). The dose × test interaction terms were not significant for line crossing [$F(3,56) = 1.9$] or wall rearing [$F(3,56) = 4.9$]. Friedman ANOVA indicated a significant exposure effect on escape attempts [$N(1,60) = 53$, $p < 0.001$] and subsequent analyses (Wilcoxon pair test) showed post-test increases in this measure in both vehicle- and drug-treated animals (0.5 to 2 mg/kg).

Flumazenil: ANOVA did not reveal a significant main effect of flumazenil on line crossing [$F(3,56) = 0.73$] or wall rearing [$F(3,56) = 1.54$] but indicated a significant overall effect with respect to escape attempts [$H = 8.69$]. Post-hoc analyses indicated that flumazenil significantly decreased escape attempts at 10 and 20 mg/kg [Mann-Whitney: $p < 0.05$ versus control]. Wall rearing [$F(1,56) = 0.29$] was not changed in the post-rat period when compared to the initial 3 min free running session. By contrast, line crossing [$F(1,56) = 6.47$, $p < 0.02$] and escape attempts [Wilcoxon pair test: $p < 0.0001$] in-

creased significantly in the post-test period. The dose × test interactions were not significant for line crossing [$F(3,56) = 1.3$] or wall rearing [$F(3,56) = 0.04$]. Friedman ANOVA indicated an overall main effect on escape attempts [Friedman: $N(1,60) = 46$, $p < 0.001$). Subsequent Wilcoxon pair test analyses indicated that escape attempts increased in all groups.

Ro 19-4603: There were significant effects of dose on frequency of line crossing [$F(3,56) = 4.24$, $p < 0.009$], wall rearing [$F(3,56) = 3.21$, $p < 0.03$] and escape attempts [$H = 18.91$, $p < 0.0003$]. Post-hoc analyses indicated reliably fewer line crossings [Newman-Keuls: $p < 0.04$ versus control] at 0.05 and 0.1 mg/kg, and significantly fewer wall rearings and escape attempts from 0.025 to 0.1 mg/kg [$p < 0.02$ versus control]. All behavioral measures were significantly affected by the predator exposure [line crossing: $F(1,56) = 19.86$, $p < 0.001$; wall rearing: $F(1,56) = 39.68$, $p < 0.001$; escape attempts: Wilcoxon pair test: $p < 0.001$]. The dose × test interactions were not significant for line crossing [$F(3,56) = 0.09$] or wall rearing [$F(3,56) = 0.44$]. For escape attempts Friedman ANOVA indicated an overall main effect of exposure [$N(1,60) = 39.09$, $p < 0.001$]. Subsequent Wilcoxon pair test analyses revealed post-test increases in escape attempts in both saline- and Ro 19-4603-treated animals.

*Predator avoidance test (Fig. 5).* Chlordiazepoxide: there were significant main effects for the number of avoidances [$H = 12.93$, $p < 0.005$] and the avoidance distance [$F(3,51) = 4.26$, $p < 0.092$], and Newman-Keuls comparisons confirmed a decrease in each measure at 25 mg/kg. Ro 19-8022: ANOVA revealed a significant main effect for the number of avoidances [$H = 12.16$, $p < 0.007$] and the avoidance distance [$F(3,46) = 3.68$, $p < 0.02$]. Subsequent post-hoc analyses with the Mann-Whitney U-test indicated that in response to an approaching predator, mice treated with Ro 19-8022, at all doses tested, showed fewer avoidance responses. Newman-Keuls comparisons revealed that the drug reduced the prey-predator distance at 0.5 mg/kg.

Flumazenil: overall ANOVA indicated a significant effect with respect to the avoidance distance [$F(3,50) = 6.45$, $p < 0.0009$], but failed to reveal a significant effect on the number of avoidances [$H = 2.96$]. Newman-Keuls analyses showed that flumazenil at 20 mg/kg significantly increased the predator-subject distance at which avoidance occurred.

Ro 19-4603: ANOVA failed to show a significant drug effect on avoidance frequency [$H = 2.53$], but revealed an overall effect with respect to the predator–subject distance at which avoidance occurred [$F(3,53) = 2.84$, $p < 0.05$]. The latter measure was reliably increased at the lowest dose (0.025 mg/kg).

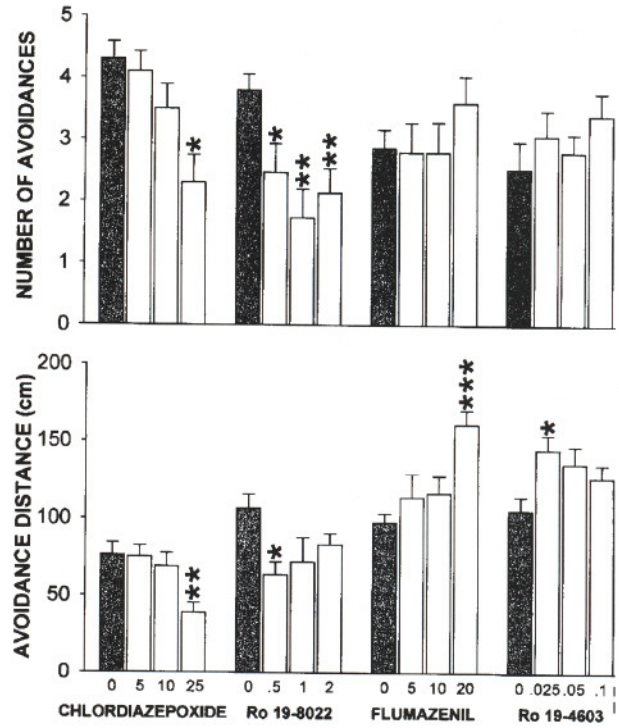


FIG. 5. Runway measures of avoidance to an approaching predator for mice administered chlordiazepoxide, Ro 19-8022, flumazenil and Ro 19-4603. Columns and vertical bars represent means and S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

*Flight/predator orientation test (Table III).* Chlordiazepoxide: ANOVA indicated a significant drug effect on overall [$F(3,55) = 4.24$, $p < 0.001$] and maximum flight speed [$F(3,55) = 16.62$, $p < 0.001$], arrests in movement [$F(3,55) = 4.24$, $p < 0.001$] and orientation to the predator [Kruskal-Wallis: $H = 27.25$, $p < 0.001$]. Post-hoc analysis indicated that the drug significantly reduced orientations and arrests at all doses tested while speed was decreased only at 10 and 25 mg/kg.

Ro 19-8022: ANOVA indicated a significant effect of drug treatment on all behavioral measures: overall flight speed [$F(3,53) = 4.99$, $p < 0.005$], maximum flight speed [$F(3,53) = 9.78$, $p < 0.001$], stops [$H = 26.48$, $p < 0.001$] and orientation to the predator [$H = 15.96$, $p < 0.002$]. Subsequent post-hoc analyses showed that Ro 19-8022 reliably increased overall flight speed at 2 mg/kg, while markedly decreasing maximum flight speed at all doses (0.5 to 2 mg/kg). Mann-Whitney U-tests indicated that the drug tended to reduce frequencies of stops and orientations to the predator at all doses.

Flumazenil: none of the behavioral responses in this test were significantly modified by flumazenil: overall flight speed [$F(3,52) = 2.06$], maximum flight speed [$F(3,52) = 1.01$], stops [$H = 5.74$] and orientation to the predator [$H = 1.89$].

Table III. Effects of chlordiazepoxide, Ro 19-8022, flumazenil and Ro 19-4603 on behavioral responses of mice chased by a predator[1]

| Dose (mg/kg) | Overall speed (m/s) | Maximum speed (m/s) | Frequency of stops | Frequency of orientations |
|---|---|---|---|---|
| Chlordiazepoxide | | | | |
| 0 | 0.54 ± 0.05 | 0.80 ± 0.05 | 9.27 ± 1.87 | 2.70 ± 0.61 |
| 5 | 0.66 ± 0.05 | 0.80 ± 0.04 | 5.67 ± 0.84* | 1.00 ± 0.31* |
| 10 | 0.65 ± 0.03 | 0.60 ± 0.04† | 3.67 ± 0.58† | 0.30 ± 0.27‡ |
| 25 | 0.49 ± 0.03 | 0.50 ± 0.03‡ | 4.07 ± 0.44† | 0.00 ± 0.00‡ |
| Ro 19-8022 | | | | |
| 0 | 0.44 ± 0.03 | 1.25 ± 0.07 | 12.40 ± 1.29 | 6.73 ± 1.00 |
| 0.5 | 0.55 ± 0.04 | 0.88 ± 0.06‡ | 6.27 ± 0.80‡ | 2.93 ± 0.41† |
| 1 | 0.54 ± 0.02 | 0.91 ± 0.04‡ | 5.54 ± 0.65‡ | 2.54 ± 0.31† |
| 2 | 0.62 ± 0.03‡ | 0.95 ± 0.05‡ | 4.57 ± 0.50‡ | 2.36 ± 0.40† |
| Flumazenil | | | | |
| 0 | 0.57 ± 0.04 | 1.23 ± 0.04 | 10.87 ± 1.69 | 4.67 ± 0.57 |
| 5 | 0.64 ± 0.06 | 1.31 ± 0.08 | 9.08 ± 1.44 | 5.00 ± 0.78 |
| 10 | 0.68 ± 0.07 | 1.23 ± 0.13 | 6.64 ± 1.06 | 3.57 ± 0.59 |
| 20 | 0.77 ± 0.06 | 1.41 ± 0.08 | 6.40 ± 1.01 | 4.53 ± 0.92 |
| Ro 19-4603 | | | | |
| 0 | 0.59 ± 0.05 | 1.19 ± 0.06 | 9.67 ± 1.05 | 4.53 ± 0.70 |
| 0.025 | 0.51 ± 0.06 | 1.18 ± 0.09 | 13.80 ± 1.13* | 8.47 ± 1.09† |
| 0.05 | 0.60 ± 0.06 | 1.17 ± 0.06 | 9.20 ± 1.20 | 4.40 ± 0.83 |
| 0.1 | 0.44 ± 0.03 | 1.02 ± 0.05 | 13.93 ± 1.08† | 6.87 ± 0.83* |

[1]Data are presented as means ± S.E.M. *$p < 0.05$, †$p < 0.01$ and ‡$p < 0.0001$.

Ro 19-4603: ANOVA failed to indicate a significant effect of drug treatment on either speed measure [overall flight speed: $F(3,56) = 2.47$; maximum flight speed: $F(3,56) = 1.58$], but revealed a significant difference with respect to stops [$H = 12.8$, $p < 0.01$] and orientations [$H = 13.14$, $p < 0.005$]. Subsequent Mann-Whitney U-test analyses indicated that Ro 19-4603 significantly increased responses at both 0.025 and 0.1 mg/kg.

*Predator approach: Straight alley (Table IV).* Chlordiazepoxide: ANOVA failed to reveal any significant main effects of treatment with chlordiazepoxide [fre-

TABLE IV. Effects of chlordiazepoxide, Ro 19-8022, flumazenil and Ro 19-4603 in the straight alley on behavioral reactions to a predator which remains at constant distance from the subject[1]

| Dose (mg/kg) | Frequency of approaches/ withdrawals | Closest distance between animals (cm) | Immobility time (s) |
|---|---|---|---|
| Chlordiazepoxide | | | |
| 0 | 3.70 ± 1.00 | 107.50 ± 13.70 | 9.50 ± 2.20 |
| 5 | 3.20 ± 0.70 | 118.10 ± 8.80 | 8.50 ± 1.10 |
| 10 | 2.10 ± 0.50 | 111.50 ± 13.70 | 13.90 ± 2.70 |
| 25 | 1.60 ± 0.40 | 127.10 ± 12.30 | 15.70 ± 2.80 |
| Ro 19-8022 | | | |
| 0 | 2.00 ± 0.31 | 150.07 ± 8.56 | 8.08 ± 1.40 |
| 0.5 | 2.73 ± 0.43 | 116.13 ± 17.26 | 7.98 ± 3.23 |
| 1 | 2.73 ± 0.51 | 119.07 ± 17.40 | 8.97 ± 2.30 |
| 2 | 2.27 ± 0.48 | 115.33 ± 18.34 | 12.81 ± 2.40 |
| Flumazenil | | | |
| 0 | 2.93 ± 0.52 | 125.87 ± 13.52 | 8.23 ± 1.58 |
| 5 | 2.33 ± 0.32 | 134.87 ± 11.40 | 6.34 ± 1.83 |
| 10 | 2.87 ± 0.53 | 130.13 ± 12.42 | 7.69 ± 1.48 |
| 20 | 2.40 ± 0.31 | 132.60 ± 10.75 | 11.57 ± 2.77 |
| Ro 19-4603 | | | |
| 0 | 2.40 ± 0.46 | 119.47 ± 16.43 | 8.21 ± 2.10 |
| 0.025 | 2.73 ± 0.40 | 128.33 ± 14.54 | 9.04 ± 2.49 |
| 0.05 | 1.80 ± 0.38 | 165.13 ± 9.07* | 13.98 ± 2.99 |
| 0.1 | 1.13 ± 0.24* | 171.80 ± 8.29* | 11.06 ± 3.23 |

[1]Data are presented as means ± S.E.M. *$p < 0.05$.

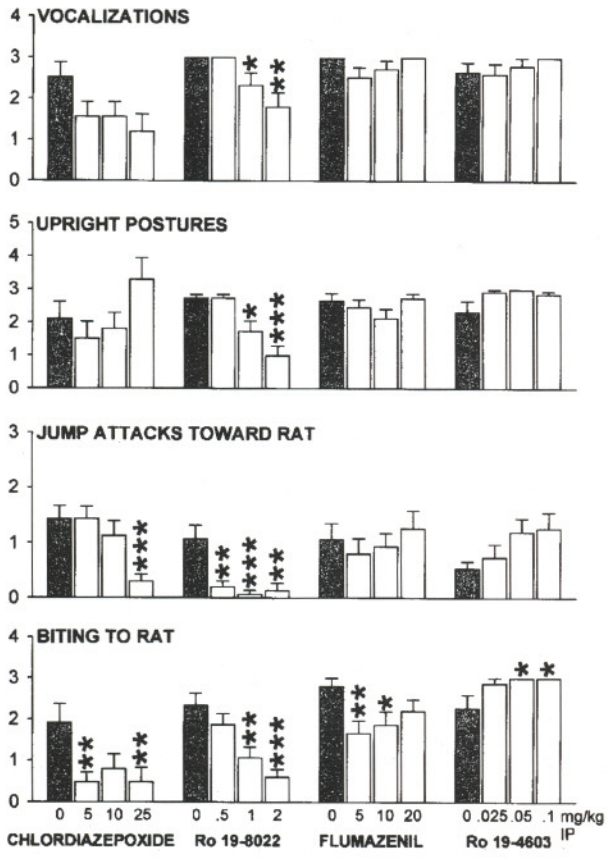


FIG. 6. Mean frequency of biting, defensive threat vocalization, upright posture and jump attacks to forced contact with a deeply anesthetized rat for subjects under varying doses of chlordiazepoxide, Ro 19-8022, flumazenil and Ro 19-4603. Columns and vertical bars represent means and S.E.M. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

quency of approaches/withdrawals: H = 5.22; closest distance between animals: F(3,55) = 0.47; immobility time: F(3,55) = 2.21].

Ro 19-8022: ANOVA failed to indicate any significant effects of the drug for frequency of approaches/withdrawals [H = 1.84], the closest distance between animals [F(3,56) = 1.1] or immobility time [F(3,56) = 0.89].

Flumazenil: none of these measures was significantly affected by the drug: approaches/withdrawals [H = 0.59], closest distance between subject and predator [F(3,56) = 0.1] and immobility time [F(3,56) = 1.26].

Ro 19-4603: ANOVA indicated a significant main effect for frequency of approaches/withdrawals [H = 9.3, $p<0.03$] and the closest distance between animals [F(3,56) = 4.32, $p<0.009$], but failed to reveal any significant action of Ro 19-4603 on immobility time [F(3,56) = 0.92]. Post-hoc analyses showed that the drug reliably decreased approaches/withdrawal activity at 0.1 mg/kg and markedly increased prey–predator distance at 0.05 and 0.1 mg/kg.

*Forced contact with the predator (Fig. 6).* Chlordiazepoxide: ANOVA indicated significant effects for frequency of biting the rat [H = 14.54, $p<0.002$] and jump attack (H = 11.05, $p<0.01$) but not for vocalization (H = 5.62) and upright posture (H = 2.8). Subsequent Mann-Whitney U-tests revealed significant reductions in biting at 5 and 25 mg/kg and in jump attack at 25 mg/kg ($p<0.001$).

Ro 19-8022: ANOVA indicated significant effects for all behavioral responses: frequency of vocalizations [H = 17.69, $p<0.001$]; occurrence of upright postures [H = 22.53, $p<0.001$); jump attacks toward the predator [H = 20.05, $p<0.001$); and frequency of biting the rat [H = 18.47, $p<0.001$). Subsequent Mann-Whitney U-tests revealed significant reductions in biting, upright posture and vocalization at 1 and 2 mg/kg, and in jump attacks at all doses (0.5 to 2 mg/kg).

Flumazenil: ANOVA indicated a significant effect for frequency of biting [H = 10.11, $p<0.02$] which subsequent Mann-Whitney U-tests showed to be due to a marked reduction of this response at 5 and 10 mg/kg. ANOVA failed to reveal significant main effects for any of the other behavioral measures: vocalization [H = 7.94]; upright posture [H = 5.09]; and jump attack [H = 1.35].

Ro 19-4603: ANOVA indicated a significant main effect for the frequency of biting [H = 12.44, $p<0.01$], but not for the occurrence of vocalization [H = 2.33]; upright posture [H = 6.01]; or jump attack [H = 6.25]. Mann-Whitney U-tests indicated a reliable increase in biting at the two highest doses of Ro 19-4603 (0.05 and 0.1 mg/kg).

*Ledge test (Table 5).* $\chi^2$ analysis indicated a significant increase in the frequency of falls at 10 and 25 mg/kg chlordiazepoxide. This measure was not significantly altered by any of the other drugs.

## DISCUSSION

In the present study, the behaviors displayed in response to rat stimuli in the oval runway are consonant with those obtained earlier, and provide confirmation of previous behavioral findings with this test (Griebel *et al.*, 1995a,b). Thus, in the contextual defense situation, post-predator escape attempts from the runway cage were dramatically increased, when compared to the performances measured before the confrontation with the rat. Similarly, in response to an approaching rat, saline-treated mice invariably showed active flight behavior, and when subjects ran to escape the chasing rat, they frequently showed risk assessment consisting of an abrupt movement arrest often followed by orientation to the oncoming rat. Furthermore, when mice were constrained in one part

TABLE V. Number of subjects showing fall from the ledge after administration of various doses of chlordiazepoxide, Ro 19-8022, flumazenil and Ro 19-4603[1]

| Dose (mg/kg) | Number of falls | *n* |
|---|---|---|
| Chlordiazepoxide | | |
| 0 | 0 | 15 |
| 5 | 2 | 15 |
| 10 | 4* | 15 |
| 25 | 4* | 14 |
| Ro 19-8022 | | |
| 0 | 0 | 15 |
| 0.5 | 1 | 15 |
| 1 | 3 | 15 |
| 2 | 4 | 15 |
| Flumazenil | | |
| 0 | 0 | 15 |
| 5 | 1 | 15 |
| 10 | 2 | 15 |
| 20 | 2 | 15 |
| Ro 19-4603 | | |
| 0 | 0 | 15 |
| 0.025 | 1 | 15 |
| 0.05 | 0 | 15 |
| 0.1 | 2 | 15 |

[1] *$p < 0.05$.

of the runway, they often displayed active risk assessment, consisting of approaches to the rat, followed by withdrawals. Finally, defensive threat and attack to the rat almost invariably occurred upon forced contact.

When compared to mice approached by a leather glove, animals confronted with an anesthetized or a conscious rat displayed more intense flight responses and defensive threat/attack reactions, while risk assessment performances were generally similar in all three conditions. Furthermore, escape attempt responses following removal of the stimulus were higher in the conscious rat condition compared to the two other groups. With the exception of these latter responses, performance of mice exposed to both rat stimuli were similar. Taken together, these results suggest that flight reactions and defensive threat/attack responses of Swiss-Webster mice confronted with a rat are specific to the latter, and thus indicate that the oval runway paradigm may be presented as one relating to 'antipredator' defense. This view is supported by several findings indicating that mice are defensive to rats and that mouse-hunting or mouse-killing are innate in laboratory rats. For instance, de Catanzaro (1988) demonstrated that inseminated female mice housed with (non-assaultive) rats produced many fewer litters, and that even the odor of the rat reduced births by increasing spontaneous abortions. Furthermore, muricide in rats does not depend on exposure to other mouse-killing rats, although mouse-eating may be facilitated by seeing another do it (Rylov and Kozyrev, 1985). Moreover, mouse-killing goes up in food-deprived rats (Rylov, 1985). Finally, predatory aggression is not changed over many generations of selection of wild Norway rats, with the selection based on presence or absence of defensive threat and attack toward human handling (Nikulina, 1991).

In addition, given that it is maladaptive to fail to show an 'antipredator' defense response in the presence of a predator, but not particularly maladaptive (i.e. simply a waste of energy) to show an 'antipredator' defense when the confronting stimulus is not a predator, one would expect 'antipredator' defensive behaviors to be very broadly coded (size, movement, eye spots). The present findings on high risk assessment performances, which are quite similar in all stimulus groups, are consonant with this view. Nonetheless, the fact that rat stimuli alone elicited avoidance responses and defensive threat/attack reactions also suggests that rats recognize some specific threat stimuli as well as those (see above) that generally tend to elicit defense.

### Motoric effects: measures of sedation and myorelaxation

Line crossings, maximum flight speed, and falls from the median wall provide data indicative of potential sedative and/or myorelaxant properties of drugs. The maximum flight speed measure suggested that the two higher doses of chlordiazepoxide may have produced such effects. This view is strongly supported by findings of falls from the partition wall at both higher doses of the drug. Because anxiolysis may also involve reduced fear of falling from the ledge, such that falls might reflect anxiety reduction rather than myorelaxant or sedative effects, we considered only those dose levels that produced both a reduction in the speed measure and an increase in the fall measure (i.e. 10 and 25 mg/kg) to be myorelaxant. In contrast to the good agreement between these two measures, line crossings were not consistently decreased for dosed as opposed to vehicle control animals, suggesting a clear dissociation of the dose levels at which myorelaxant and sedative effects occurred.

With respect to the other compounds tested in the present study, evidence for myorelaxant effects was minimal, with only Ro 19-8022 reducing maximum flight speed at all doses, and no other drug producing an increase in falls. Also, as discussed below, the effect of Ro 19-8022 on maximum flight speed may reflect a disinhibitory action of the drug rather than myorelaxation.

In addition, the findings that Ro 19-8022 (at 1 and 2 mg/kg) and chlordiazepoxide (at 10 mg/kg) reliably increased line crossing measure, tend to indicate that these effects may be attributed to a fear/anxiety-reducing action of both drugs. Consonant with this view, we recently demonstrated that chlordiazepoxide and Ro 19-8022, administered to BALB/c mice, dramatically increased horizontal and vertical motor patterns while reversing their neophobic reactions toward an unfamiliar

area (Griebel *et al.*, 1993). Recent data from the mouse defense test battery (MDTB) showed that the 5-$HT_{1A}$ receptor partial agonist and anti-anxiety agent gepirone, as well as the 5-$HT_{1A}$ receptor full agonist 8-OH-DPAT, strongly decreased spontaneous horizontal activity, while imipramine and fluoxetine, given repeatedly, failed to affect this response (Griebel *et al.*, 1995a,b). Taken together, these findings suggest that line crossing measure in this paradigm might be useful to differentiate between the action of BZ receptor agonists and those of other anxiolytics, in particular 5-HT interacting drugs. Further studies are needed, however, in order to confirm the generality of these preliminary findings.

### Drug effects preceding and following rat exposure: 'contextual defense'

In previous studies using the MDTB, the 5-$HT_{1A}$ anti-anxiety agent gepirone affected the potentiation of escape attempts (which were divided into wall climbing plus jump escapes in that first study using the MDTB, but which we have recombined for comparison with the present results) after rat exposure (Griebel *et al.*, 1995b), while chronic treatments with the antipanic drugs imipramine and fluoxetine were unable to counteract the post-rat increase in these responses (Griebel *et al.*, 1995a). In the present study, the BZ agonists chlordiazepoxide and Ro 19-8022 failed to alter the enhancement of escape attempts following rat exposure. In contrast, both Ro 19-4603 and flumazenil produced reliable reductions in the frequency of escape attempts following rat exposure, compared to those of saline-treated animals. Ro 19-4603 also reduced both wall rearings and (at the two higher doses) line crossings, suggesting the possibility of hypoactivity. In two earlier studies Ro 19-4603, at similar doses, had no effect on locomotion in a familiar environment (Belzung *et al.*, 1990; Jackson and Nutt, 1992), but the 0.1 mg/kg dose of Ro 19-4603 reduced transitions in an unfamiliar light/dark box (Belzung *et al.*, 1990). Thus it is unclear whether the present contextual defense effects of Ro 19-4603 primarily reflect some relatively general reduction in locomotion, or whether emotionality changes are involved. Flumazenil had no apparent effect on line crossings or wall rearings, counterindicating a general activity reduction or suppression. Thus the reduction in post-test escape attempts at 10 and 20 mg/kg flumazenil, compared to those of saline-treated animals, suggests a direct modulation of defensive behavior in the post-rat context.

### Drug effects during exposure to the rat

*Flight.* Recent data from the MDTB have clearly demonstrated that panic-modulating agents specifically affect animals' flight responses. Thus, the panic-promoting drug yohimbine has been found to increase prey-predator distance at which flight occurred (Blanchard *et al.*, 1993a), while the antipanic agents imipramine and fluoxetine, given repeatedly, strongly reduced flight behaviors. Furthermore, gepirone failed to affect this response in a selective manner (i.e. at non sedative doses) (Griebel *et al.*, 1995a,b). In light of the suggestion that panic symptoms are due to pathological and spontaneous activation of neuronal mechanisms underlying flight reactions (Graeff, 1990; Deakin and Graeff, 1991; Deakin *et al.*, 1991), we concluded that the MDTB provides measures that serve as an effective experimental model of panic attack.

In the present situation, chlordiazepoxide failed to reduce avoidance distance or frequency at non sedative/myorelaxant level. Such an effect is in agreement with the low potency of the drug to alleviate panic attacks at anxiolytic doses. By contrast, Ro 19-8022 strongly decreased flight/avoidance responses at all doses (0.5 to 2 mg/kg) while the 0.5 mg/kg dose also reduced the distance between the subject and the predator at which flight occurred. The compound also consistently reduced maximum flight speed in the chase/flight test. Since these effects were obtained at levels at which no evidence of an increase in falls was obtained, the action of this BZ partial agonist reflects specific reductions in activation of defense-related flight systems. Taken together, these data strongly suggest that Ro 19-8022 may possess considerable potency as therapeutic agents for panic disorder. Nevertheless, in a previous study using the MDTB (Griebel *et al.*, 1995a), neither imipramine nor fluoxetine given repeatedly significantly affected maximum flight speed, suggesting that flight/avoidance measures but not maximum flight speed may be considered as a relevant index of panic related symptoms.

In contrast, both flumazenil (20 mg/kg) and Ro 19-4603 (0.025 mg/kg) reliably increased avoidance distance. This anxiogenic-like action of flumazenil is in agreement with clinical reports indicating that the drug is somewhat anxiogenic in volunteers (Darragh *et al.*, 1983; Schopf *et al.*, 1984; Duka *et al.*, 1986; Higgitt *et al.*, 1986; Lavie, 1987) and, especially, that it increases the frequency of panic attacks in panic disorder patients (Nutt *et al.*, 1990). For Ro 19-4603, this increase in the predator–subject distance leading to avoidance is consonant with an anxiogenic-like action recently observed in the mouse light/dark choice paradigm (Belzung *et al.*, 1990).

*Risk assessment.* Our earlier MDTB studies with the 5-$HT_{1A}$ receptor agonists 8-OH-DPAT and gepirone revealed that both compounds markedly reduced risk assessment activities in the straight alley situation, while neither affected similar responses (i.e. orientations and

stops) during the chase/flight test (Griebel *et al.*, 1995b). By contrast, long-term treatments with imipramine and fluoxetine failed to alter the former, while markedly reducing risk assessment activities when the subject was chased (Griebel *et al.*, 1995a). In the present study, the results obtained with chlordiazepoxide and Ro 19-8022 closely resemble those observed with both imipramine and fluoxetine, while mice treated with the BZ inverse agonist Ro 19-4603 showed an opposite effect (significantly more stops and orientations to the predator) during the chase/flight test. However, Ro 19-4603 at the highest dose also reduced risk assessment (frequency of approach/withdrawal to the predator) in the straight alley situation, accompanied by an increase in the minimal prey–predator distance (0.05 and 0.1 mg/kg). Since approach/withdrawal is an active behavior, while stops (although not orientation) might actually be enhanced by hypoactivity, it is possible that the high dose reductions in approach/withdrawal, like the reductions in all of the contextual defense responses at these levels, may largely reflect some form of locomotor hypoactivity. However, based on the pattern of findings, including a failure to find reductions in overall speed or maximum speed in this situation, and the apparent relationship between hypoactivity and novelty/threat noted in other studies for subjects given Ro 19-4603 (Belzung *et al.*, 1990; Jackson and Nutt, 1992), a simple hypoactivity interpretation of the present Ro 19-4603 effects is dubious. An alternative explanation might be that responses to highly threatening stimuli (i.e. an approaching rat) may involve central mechanisms that can override the strong hypolocomotor effects seen in the contextual defense situation, in which there is no discrete threat stimulus and levels of defensiveness are undoubtedly lower. Finally, none of the subjects' risk assessment responses were substantially affected by the administration of flumazenil, indicating that in this particular situation the drug displayed no intrinsic activity.

*Defensive threat and attack.* When contact was forced between predator and subject, the administration of chlordiazepoxide and Ro 19-8022 markedly reduced defensive attack and vocalizations, upright postures, and frequency of biting the rat. This reduced defensive threat/attack profile is very similar to that observed in previous studies in rats with BZ full agonists (Blanchard *et al.*, 1989), suggesting that these defensive responses are sensitive to anxiolytic drugs. Surprisingly, flumazenil at the two lowest doses (5 and 10 mg/kg) also inhibited biting responses, thereby displaying an agonist- rather than an inverse agonist-like profile in this particular situation. Finally, the administration of the BZ inverse agonist Ro 19-4603 potentiated subjects' biting responses at 0.05 and 0.1 mg/kg. Compared to the action of BZ agonists on biting, Ro 19-4603 produced an opposite, enhanced, defensive behavior, thus confirming the overall anxiogenic-like profile of Ro 19-4603 in the MDTB.

In summary, these findings provide strong evidence for an anxiolytic-like action of the BZ full agonist chlordiazepoxide and the BZ partial agonist Ro 19-8022 in the MDTB. Many specific behavioral changes, including reductions in risk assessment (stops and orientations) in the chase/flight test, and reductions in defensive threat/attack to approach and contact by the predator, were consonant with mouse and rat findings in very similar test situations (Blanchard *et al.*, 1990; 1993b). However, some effects of the BZ partial agonist, such as reduced frequency of avoidance and shorter rat–subject distance required to elicit avoidance, were not seen with BZ full agonists in the MDTB. However, this pattern is characteristic of compounds with efficacy against panic disorder (Griebel *et al.*, 1995a).

In contrast, mice treated with the BZ panic disorder inverse agonist Ro 19-4603 displayed an overall anxiogenic-like profile, with one or more dose levels producing the opposite effect to Ro 19-8022 on the same measures (stops and orientations in the chase/flight test, and biting the rat) on which Ro 19-8022 effects were both reliable and identical to those of the BZ full agonist. Although the effect on contextual escape attempts might reflect a general behavioral suppression, it seems that the action of the drug on flight and risk assessment activities is compatible with an interpretation of enhanced defensiveness.

As expected, the administration of the BZ antagonist flumazenil was without effect on most of these measures. Moreover, the effects obtained were varied: it produced a BZ agonist-like activity, i.e. fewer bites, in a highly threatening situation (i.e. when contact was forced with the predator), but increased predator–subject distance needed to elicit avoidance, an effect seen also with the BZ inverse agonist, to an approaching predator. These findings afford a tentative suggestion that the immediacy/intensity of the threat stimulus may be an important factor in determining if the (at best weak) action of flumazenil at the BZ receptor is agonist-like, or more similar to that of an inverse agonist.

Finally, these results agree with previous analyses indicating that BZ receptor ligands consistently affect only a subset of defensive behaviors, and permit a reassessment of what behaviors fall into this group. Predator- or risk-assessment behaviors, the central feature of an 'anxiolytic profile' identified in the rat defensive response to a variety of anxiolytic compounds (Blanchard *et al.*, 1993b), appear to show changes similar to those produced by BZ full and partial agonists, and the opposite effect to BZ inverse agonists; a similar pattern was obtained for defensive biting of the predator. Other behaviors, including flight/avoidance related measures, were

reduced here in response to the BZ partial agonists and enhanced by the inverse agonist, but are reduced only at sedative doses, in response to BZ full agonists. Furthermore, the present contextual defense measures were not responsive to the BZ partial agonist, nor, at nonsedative doses, to the BZ full agonists. These results thus indicate that risk assessment behaviors, and biting, show a consistent and appropriate response to BZ receptor full agonists, partial agonists, and inverse agonists, and suggest a hypothesis that the action of the BZ is particularly involved in modulation of these behaviors. Finally, taken together with previous experiments with the MDTB using serotonergic anti-anxiety agents, the present findings strongly suggest that this paradigm is able to differentiate between different classes of anxiolytics.

## Acknowledgement

This research was supported by USPHS Awards NIH MH42803, and RR03061. Guy Griebel was supported by funds from Institut de Recherches Internationales Servier (Courbevoie, France) and from the Association pour la Défense des Sciences du Comportement (Strasbourg, France). We thank Dr J.-L. Moreau and Dr U. Fischer (F. Hoffmann-La Roche Ltd., Basel, Switzerland) for the generous gifts of Ro 19-8022, flumazenil and Ro 19-4603.

## REFERENCES

Belzung C, Misslin R and Vogel E (1990) Anxiogenic effects of a benzodiazepine receptor partial inverse agonist, Ro 19-4603, in a light/dark choice situation. *Pharmacology Biochemistry and Behavior*, **36**, 593–596.

Blanchard DC and Blanchard RJ (1990) Effects of ethanol, benzodiazepines and serotonin compounds on ethopharmacological models of anxiety. In: *Anxiety* (Eds N McNaughton and G Andrews), pp. 188–199. University of Otago Press, Dunedin.

Blanchard DC, Hori K, Rodgers RJ and Hendrie CA (1989) Attenuation of defensive threat and attack in wild rats (*Rattus rattus*) by benzodiazepines. *Psychopharmacology (Berlin)*, **97**, 392–401.

Blanchard DC, Blanchard RJ, Tom P and Rodgers RJ (1990) Diazepam changes risk assessment in an anxiety/defense test battery. *Psychopharmacology (Berlin)*, **101**, 511–518.

Blanchard RJ, Taukulis HK, Rodgers RJ, Magee LK and Blanchard DC (1993a) Yohimbine potentiates active defensive responses to threatening stimuli in Swiss-Webster mice. *Pharmacology Biochemistry and Behavior*, **44**, 673–681.

Blanchard RJ, Yudko EB, Rodgers RJ and Blanchard DC (1993b) Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. *Behavioural Brain Research*, **58**, 155–165.

Blanchard DC, Rodgers RJ, Shepherd JK and Blanchard RJ (1994) Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. In: *Ethopharmacology of Aggression and Defense: Benzodiazepine and Serotonergic Effects* (Eds KA Miczek and SE Cooper). Oxford University Press, Oxford (in press).

Braestrup C, Honore T, Nielsen M, Petersen EN and Jensen LH (1984) Ligands for benzodiazepine receptors with positive and negative efficacy. *Biochemical Pharmacology*, **33**, 859–862.

Darragh A, Lambe R, O'Boyle C, Kenny M and Brick I (1983) Absence of central effects in man of the benzodiazepine antagonist Ro 15-1788. *Psychopharmacology (Berlin)*, **80**, 192–195.

De Catanzaro D (1988) Effect of predator exposure upon early pregnancy in mice. *Physiology and Behavior*, **43**, 691–696.

Deakin JFW and Graeff FG (1991) 5-HT and mechanisms of defense. *Journal of Psychopharmacology*, **5**, 305–315.

Deakin JFW, Guimaraes FS, Wang F, Hellewell J and Hensman R (1991) 5-HT receptor mechanisms in human anxiety. In: *New Concepts in Anxiety* (Eds M Briley and SE File), pp. 74–93. CRC Press, New York.

Dorow R, Horowski R, Paschelke G and Amin M (1983) Severe anxiety induced by FG 7142, a beta-carboline ligand for benzodiazepine receptors [letter]. *Lancet*, **2**, 98–99.

Duka T, Ackenheil M, Noderer J and Doenicke A (1986) Changes in noradrenaline plasma levels and behavioural responses induced by benzodiazepine agonists with the benzodiazepine antagonist Ro 15-1788. *Psychopharmacology (Berlin)*, **90**, 351–357.

Facklam M, Schoch P, Bonetti EP, Jenck F, Martin JR, Moreau JL and Haefely WE (1992) Relationship between benzodiazepine receptor occupancy and functional effects in vivo of four ligands of differing intrinsic efficacies. *Journal of Pharmacology and Experimental Therapeutics*, **261**, 1113–1121.

File SE, Lister RG and Nutt DJ (1982) The anxiogenic action of benzodiazepine antagonists. *Neuropharmacology*, **21**, 1033–1037.

Gentil V, Tavares S, Gorenstein C and Bello C (1990) Acute reversal of flunitrazepam effects by Ro 15-1788 and Ro 15-3505: inverse agonism, tolerance, and rebound. *Psychopharmacology (Berlin)*, **100**, 54–59.

Graeff FG (1990) Brain defense system and anxiety. In: Handbook of Anxiety. Vol. 3: *The Neurobiology of Anxiety* (Eds GD Burrows, M Roth, and R Noyes, Jr.), pp. 307–343. Elsevier, Amsterdam.

Griebel G, Belzung C, Misslin R and Vogel E (1993) The free-exploratory paradigm: an effective method for measuring neophobic behaviour in mice and testing potential neophobia-reducing drugs. *Behavioural Pharmacology*, **4**, 637–644.

Griebel G, Blanchard DC, Agnes RS and Blanchard RJ (1995a) Differential modulation of antipredator defensive behavior following acute or chronic administration of imipramine and fluoxetine. *Psychopharmacology (Berlin)* (in press).

Griebel G, Blanchard DC, Jung A, Masuda CK and Blanchard RJ (1995b) 5-$HT_{1A}$ agonists modulate mouse antipredator defensive behavior differently than the 5-$HT_{2A}$ antagonist pirenperone. *Pharmacology, Biochemistry and Behavior* (in press).

Haefely W (1983) Antagonists of benzodiazepines: functional aspects. *Advances in Biochemical Psychopharmacology*, **38**, 73–93.

Haefely WE (1989) Pharmacology of the benzodiazepine receptor. *European Archives of Psychiatry and Clinical Neuroscience*, **238**, 294–301.

Higgitt AC, Lader MH and Fonagy P (1986) The effects of the benzodiazepine antagonist Ro 15-1788 on psychophysiological performance and subjective measures in normal subjects. *Psychopharmacology (Berlin)*, **89**, 395–403.

Jackson HC and Nutt DJ (1992) Effects of benzodiazepine

receptor inverse agonists on locomotor activity and exploration in mice. *European Journal of Pharmacology*, **221**, 199–203.

Jenck F, Moreau JL, Bonetti EP, Martin JR and Haefely WE (1992) Ro 19-8022, a nonbenzodiazepine partial agonist at benzodiazepine receptors: neuropharmacological profile of a potential anxiolytic. *Journal of Pharmacology and Experimental Therapeutics*, **262**, 1121–1127.

Jensen LH (1984) Evaluation of the $\beta$-carboline ZK 93 426 as a benzodiazepine receptor antagonist. *Psychopharmacology (Berlin)*, **83**, 249–256.

Katschnig H, Merz WA and Berger P (1991) Attack related treatment of panic disorder with bretazenil: a randomised placebo controlled clinical trial. In: *5th World Congress of Biological Psychiatry* (Eds G Racagni, N Brunello and T Fukuda), pp. 709–712. Elsevier Science Publisher, Amsterdam.

Lavie P (1987) Ro 15-1788 decreases hypnotic effects of sleep deprivation. *Life Sciences*, **41**, 227–233.

Nikulina EM (1991) Neural control of predatory aggression in wild and domesticated animals. *Neuroscience and Biobehavioral Reviews*, **15**, 545–547.

Nutt DJ, Glue P, Lawson C and Wilson S (1990) Flumazenil provocation of panic attacks: evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Archives of General Psychiatry*, **47**, 917–925.

Pieri L (1988) A benzodiazepine receptor partial inverse antagonist with prolonged proconvulsant action in rodents. *British Journal of Pharmacology*, **95**, 477P.

Poshivalov VP (1987) Ethopharmacological and neuropharmacological analyses of agonistic behaviour. In: *Ethopharmacology of Agonistic Behaviour in Animals and Humans* (Eds B Olivier, J Mos and PF Brain), pp. 122–131. Martinus Nijhoff, Dordrecht.

Rodgers RJ (1991) Effects of benzodiazepine and 5-HT receptor ligands on aggression and defense in animals. In: *5-$HT_{1A}$ Agonists, 5-$HT_3$ Antagonists and Benzodiazepines. Their Comparative Behavioural Pharmacology* (Eds RJ Rodgers and SJ Cooper), pp. 195–231. John Wiley & Sons, Chichester.

Rylov AI (1985) Change of predator intraspecies aggression of male rats under food deprivation. *Zhurnal Vysshei Nervoi Deyatel'nosti*, **35**, 875–878.

Rylov AI and Kozyrev SA (1985) Correlation of genetic and environmental factors in manifestation of predatory reactions in rats. *Zhurnal Vysshei Nervoi Deyatel'nosti*, **35**, 480–486.

Schopf J, Laurian S, Le PK and Gaillard JM (1984) Intrinsic activity of the benzodiazepine antagonist Ro 15-1788 in man: an electrophysiological investigation. *Pharmacopsychiatry*, **17**, 79–83.

Treit D (1991) Anxiolytic effects of benzodiazepines and 5-$HT_{1A}$ agonists: animal models. In: *5-$HT_{1A}$ Agonists, 5-$HT_3$ Antagonists and Benzodiazepines. Their Comparative Behavioural Pharmacology* (Eds RJ Rodgers and SJ Cooper), pp. 107–131. John Wiley & Sons, Chichester.

Willer JC, Von Frenkell R, Bonnet D and Le Fur G (1986) The ability of PK 8165, a quinoline derivative, to reduce responses to a stressful situation in a double-blind study in man. *Neuropharmacology*, **25**, 275–281.

*(Received 3 October 1994; accepted as revised 2 April 1995)*