

# Benzodiazepine and Serotonergic Modulation of Antipredator and Conspecific Defense

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BLANCHARD, D. C., G. GRIEBEL, R. J. RODGERS AND R. J. BLANCHARD. *Benzodiazepine and serotonergic modulation of antipredator and conspecific defense*. NEUROSCI BIOBEHAV REV 22(5) 597–612.—The mammalian defense repertory comprises an array of individual behaviors that are extraordinarily sensitive to relevant features of the threat stimulus and the situation in which it occurs. In parallel with increasing awareness of the specificity and complexity of defensive behaviors and of their potential relevance to psychopathologies (e.g. anxiety, panic, and depression) is an escalating use of natural threat stimuli such as attacking conspecifics or predators in paradigms aimed at evaluating drug effects on defense. A review of the literature on benzodiazepine (BZ) and serotonin (5-HT) effects on conspecific and antipredator defense, including defensive analgesia, indicates that both types of stimuli elicit a wide array of relevant defensive behaviors. These studies suggest specificity of drug effects on particular behaviors, rather than a general alteration of all aspects of defense. However, stimulus variability and possible confounding of effects are a considerable problem with conspecific defense paradigms, while antipredator paradigms utilizing human experimenters as the predator may be difficult to use with domesticated laboratory animal subjects. In addition, sensitivity to the organization of defensive behaviors and to differences between species in defense patterns is necessary to adequate interpretation of results. Nonetheless, these paradigms have permitted major advancements in analysis of the behavioral defense systems and their sensitive use in drug studies will greatly facilitate an understanding of the physiology of defense. © 1998 Elsevier Science Ltd. All rights reserved

Benzodiazepine    Serotonin    Anxiety    Fear    Defense    Rat    Mouse    Conspecific aggression    Antipredator defense

## INTRODUCTION

WHEN A defensive response is inadequate to the situation in which it occurs the outcome is likely to be rapid and disastrous to the extended reproductive fitness of the responder. Thus, the behavioral defense systems may reasonably be regarded as the product of extraordinarily strong selection pressures affecting not only the form and magnitude of particular defensive reactions but also the relationship of each type of defensive behavior to relevant features of the threat stimuli/situation to which that defensive behavior is an adaptive response. Consonant with this view, recent analysis of the defensive repertoire for wild and laboratory rodents indicates that it comprises an array of individual behaviors (1). A number of these (e.g. flight, freezing, defensive threat and attack) correspond with behaviors which can be elicited and are differentially modifiable by site-specific brain manipulations (2–6).

This behavioral and brain system diversity suggests that involvement of different defensive behaviors may provide a potential explanation for the variety of human psychopathologies relating to defense (7–10). It further suggests the value of determining the differential effects of psychoactive compounds on each type of defensive

behavior. The present review will attempt to describe the results of test situations involving the use of “natural” threat stimuli such as attacking conspecifics and predators to elicit defense, and enabling some degree of analysis of drug effects on individual defensive behaviors rather than on a single, arbitrary or derived “defense” score or measure. While such an approach is characteristic of only a small component of the research aimed at evaluation of the effects of drugs on anxiety, it promises to provide a more detailed and specific understanding of the effects of both traditional (GABA/benzodiazepine) and novel (serotonergic) anxiolytics.

## CONSPECIFIC DEFENSE

### *Reactions to present threat stimuli*

The defensive behaviors of rats (*Rattus norvegicus*) under attack by conspecifics have been extensively described and analyzed in resident-intruder and social grouping situations. The classic studies of Grant and his co-workers (11–13) provided an excellent descriptive/analytic basis for differentiating conspecific aggression and defense patterns of rats. Except for differences in interpretation of the

functional status of particular behaviors, their classifications of conspecific defensive behaviors have been used with only relatively minor changes by most investigators over the past quarter century.

The conspecific defensive reactions of rats, in the small to medium enclosures generally used, include flight (typically brief and abortive), defensive ultrasounds (typically a mixture of 18–26 and 35–70 kHz cries), freezing between attack bouts, and specific postures and movements protecting the back, the primary target of offensive attack in this species (14,15). One of these, lying “on-the-back” is widely regarded as a “submissive” behavior inhibiting further conspecific attack (e.g. (16)). Alternatively, it is also interpreted as simply the highest level of back-defense, reducing biting by concealing the target for biting attack (17).

Mice (*Mus musculus* or *Mus domesticus*) also show flight and freezing, the latter typically either in an upright “defensive” posture or in a drooping upright “submissive” posture. An important factor in many mouse studies is that isolation over several weeks tends to strongly polarize agonistic behaviors in this species, with a subgroup of “timid” mice showing rapid and high-magnitude assumption of “submissive” postures to minimal conspecific attack, while “aggressive” mice show very persistent attack toward male conspecifics. “Timid” mice have been selected for use in research on defensiveness (18), introducing a potentially important subject-selection factor into studies analyzing drug effects in this species.

#### *Benzodiazepine (BZ) effects on immediate reactions to an attacking conspecific*

##### *Rats*

In the first comprehensive study of BZ effects on conspecific defense, victorious and defeated rats were determined by three sessions in a food competition situation. In this test rats passed through a tube to obtain food, and later were started at opposite ends of the tube to meet in the middle (19). Chlordiazepoxide given to the defeated rat, now paired with another victorious male, increased (at 2.5 and 5.0 mg/kg, i.m.) both submissive-supine and defensive-upright postures, and at 10.0 and 20.0 mg/kg prolonged immobile crouching. However, victorious (undrugged) opponents also showed increases in attacks and offensive blocks, plus longer durations of aggressive posture, to defeated rats given 5.0 mg/kg, but not higher doses, of chlordiazepoxide. Thus attacker behavior may be in part responsible for the higher levels of defensiveness seen at this dose, while the highest dose used may have sedative properties enhancing immobile postures.

In contrast, in a colony intrusion paradigm (20), chronic (5-days) pretreatment of intruders with chlordiazepoxide (5 mg/kg) or lorazepam (0.25 mg/kg) significantly reduced the amount of attack to which these animals were subjected and the incidence of intruder submissive behavior. Furthermore, compared to controls, BZ-treated intruders initiated more social investigation and aggression towards colony residents. In a follow-up study, chlordiazepoxide (five days pretreatment with 5 mg/kg) was found to prevent the plasma corticosterone response of colony intruders (21). However,

as in the earlier (20) study, chlordiazepoxide-treated intruders were subjected to markedly fewer attacks than controls; as such, their reduced endocrine response may simply reflect the lower level of aversive stimulation experienced. BZ modulation of conspecific defense is also indicated by the findings of Beck and Cooper (22,23) that, in cohabiting pairs of male rats, the BZ receptor inverse partial agonist, FG 7142 (2.5–10 mg/kg), decreases aggression and increases avoidance. These effects are reversed by the BZ antagonist flumazenil (10 mg/kg) and attenuated by chlordiazepoxide (5 mg/kg), which, alone, had intrinsic effects opposite to those of FG 7142.

The possible role of differential attacker behavior on the effects of BZs on conspecific defense has been further analyzed by Piret et al. (24). In that study a BZ full (diazepam), a partial (ZK 91296), and a partial inverse agonist (FG 7142) were given to male rats used as intruders into the home cage of an attacking resident. Diazepam (chronic administration through implanted silastic tubes, allowing average release of 5 mg/kg/24 h) increased freezing frequency, duration of defensive upright, and frequency and duration of partner investigation, while decreasing frequency and duration of on-the-back. The partial agonist, ZK 91296 (at the lowest dose used, 5 mg/kg, only), decreased frequency and duration of crouching, frequency and duration of cage exploration, and increased defensive upright. The inverse agonist, FG 7142, increased frequency and duration of crouching, increased freezing frequency, and decreased frequency of defensive upright.

These patterns, as obtained, fit very well with the interpretation (24) that chronic diazepam alters the form of defense, promoting freezing and defensive upright, and reducing on-the-back “submissive” postures, with this pattern duplicated by the partial agonist and an opposite effect seen with the inverse agonist. However, the undrugged attackers confronting agonist/partial agonist-treated intruders showed a clear trend toward reduced attack, while those attacking intruders treated with the inverse agonist, showed more. When expressed as a proportion of the offensive attack received, neither ZK 91296 or FG 7142 produced any reliable effects whatever, and the significant diazepam effects were reduced to an increase in the duration of defensive upright/sideways, and a decrease in the duration of on-the-back. This last finding fits with either the view that diazepam differentially impacts “defense” vs. “submission” or, the less theoretical interpretation that it promotes a progression from more intense defensive behavior (on-the-back) to less intense elements (defensive upright/sideways). However, the striking effects of intruder treatment on attacker behavior, and the difference in reliable drug effects when attacker behavior is, or is not, incorporated into the analysis illustrate a common problem in the evaluation of drug effects during conspecific interactions; these may be due to direct effects on the treated animal, to changes in partner behavior to the treated animal, or, to some interaction of the two. In fact, analysis of the above studies of BZ effects on conspecific defense suggest considerable variation from one study to another for the same compound at similar dose levels (e.g. chlordiazepoxide; (21,19)). While these variations may reflect differences in methodology or measures taken, it is notable that when a given compound produces change in the

attacker, attacker behavior, when measured, appears to be so consistent with these attackee changes as to suggest an important indirect drug effect on defensive behavior through alteration of the behavior of the attacker.

### Mice

The possibility of differential attack to BZ-treated conspecifics may also be a problem in mice, although in contrast to the variable direction of effect in rat studies consistently increased attack has been found to BZ-treated mice. Dixon (25) has reported increased aggression toward mice smeared with urine from diazepam-drugged donors, while Borgesova et al. (26) found increased aggression to chlordiazepoxide-treated partners. Such problems are likely reduced, however, when the nondrugged resident or intruder is a selected nonattacker.

Everill et al. (27) used anosmic, group-housed non-aggressive intruders into the home cages of male mice of three strains, finding many strain differences in ten reported categories of behavior. However, CDP (2.5–10 mg/kg) systematically altered only two of these, a “defensive–submissive” category, and immobility: both showed a dose-dependent increase, a finding that appears not to reflect sedation as other, more active categories of behavior were not reduced.

Extensive research by Krsiak and his colleagues has detailed BZ effects on isolated timid mice paired with group-housed nonattackers, typically measuring “defense” as the assumption of a hunched back, raised forepaws posture in response to social investigation, and “escape” as running or jumping away from the opponent, with rearing and walking evaluated as controls for activity changes.

Sulcova and Krsiak (28) report the effects of nine BZs on these behaviors. All nine BZs (alprazolam, oxazepam, diazepam, clonazepam, nitrazepam, flunitrazepam, chlordiazepoxide, triazolam, and lorazepam; all p.o.) reduced defensive upright reliably at some of the doses given, with diazepam, alprazolam and oxazepam reducing defense at doses which did not reliably alter escape. The view that some BZs differentially impact defensive upright and escape reactions is compatible with an earlier report that, while 4.0 mg/kg diazepam reduced both defensive upright and escape, simultaneous administration of diazepam plus the inverse agonist Ethyl-B-carboline-3-carboxylate ( $\beta$ -CCE; 1.0 mg/kg) (which, alone, produced no behavioral effects), resulted in a reliable reduction in defensive upright but not escape in timid mice (29). The remaining six BZs of the Sulcova and Krsiak (28) study reduced defense and escape at similar doses, but with behavioral profiles suggesting possible sedative effects.

In a subsequent study Krsiak and Sulcova (30) generally replicated these findings with reference to the possible involvement of sedation in the defensive upright/escape effects of three 2'-chloro-phenyl-BZs (triazolam, clonazepam and lorazepam). They also repeated their finding of reduced defensive upright with three 2'-deschloro-phenyl-BZs; alprazolam, nitrazepam and oxazepam. However, in contrast to the earlier study, escape behavior was reduced reliably at doses of alprazolam, nitrazepam and oxazepam similar to those altering defensive upright. Thus, while these

data are consonant with a view that the 2'-deschloro-phenyl-benzodiazepines may have a direct effect on some elements of defensive behavior, they provide little evidence for behavioral specificity of those effects.

The support these studies provide for a differential effect of some BZs on defensive upright as opposed to escape is thus equivocal. In addition, even if there is such a differential effect, it may be characteristic of a very narrow range of doses. Sulcova and Krsiak (28) obtained such a differentiation at 3.0 mg/kg diazepam, p.o. Sulcova et al. (31) found daily 5 mg/kg doses of diazepam reduced escape as much as defense. Also, forty eight hours after the 8th and final administration, escape, but not defense, showed a reliable *increase* relative to controls. Poshivalov (32), also using isolation-timid mice, reported reductions in both defense and escape, at 3.5 mg/kg, p.o. diazepam. However, Poshivalov's aggressive male threat stimuli may have produced higher response baselines for escape and thus eliminated a floor effect for escape. In those studies reporting differential BZ effects, control escape measures were typically much lower than those for defensive upright. Also, since a 3.1 mg/kg diazepam dose has been reported (18) to impair balance on a rota-rod for singly-housed mice, balance effects seen at such levels may reduce ability to maintain an upright posture more than they alter escape responses.

Finally, the effects of BZs on defense appear to be mediated by the central-type of BZ binding site. Ro 5-4864, a 1,4-BZ which has very high affinity for the peripheral type of BZ binding site, but low affinity for the central type, produced no effect on defense in timid mice across a range of doses (2.5–10 mg/kg) (33). That central-type BZ receptors alter the response of animals to conspecific attack is consonant with the findings of increased *in vivo* [<sup>3</sup>H]Ro 15-1788 BZ binding in cerebral cortex, cerebellum and hypothalamus in defeated mice. This increase was reduced by adrenalectomy and restored by corticosterone replacement (34). Specific involvement of BZ receptor mechanisms in the inhibitory effects of BZs on defense/escape in male mice is further suggested by two lines of evidence 1), the  $\beta$ -carboline inverse partial agonists,  $\beta$ -CCE and FG 7142, stimulate defensiveness and timidity (32,35,36) and 2) the effects of BZ agonists are blocked by a range of BZ antagonists/inverse agonists including Ro15-1788 (flumazenil),  $\beta$ -CCE, FG 7142 and CGS 8216 (37,38,29,36).

### Hamsters

Further evidence of differential effects of BZ on particular components of the defense response may be found in work indicating that previously defeated male hamsters subsequently show reduced aggression and enhanced flight in response to a nonaggressive intruder into their home cages (39). DZP (2–20 mg/kg) administered either just following the initial defeat experience, or just prior to intruder testing 24 h after defeat, dose-dependently potentiated the flight response while tending to reduce defensive postures. Since these effects were of similar magnitude when the drug was given after the defeat experience and 24 h prior to testing, or just before testing, it appears likely that some type of memory or information processing mechanism may be involved in the effect.

### *5-Hydroxytryptamine (5-HT) effects on immediate reactions to an attacking conspecific*

#### *Rats*

Although rats have been extensively used in pharmacological studies of social and agonistic interactions, the major thrust of this work has been the analysis of aggressive behaviors, with defense categories often either not analyzed, or analyzed in situations in which the (aggressive) subject is unlikely to display much defense. Thus, eltoprazine (5-HT<sub>1A/1B</sub> agonist and (weak) 2C antagonist) decreased conspecific aggression by resident rats in a dose-dependent fashion (1.0–5.0 mg/kg) with no increase in defense (40). Aggression results were similar when the drug was given to sham operated or to rats with 5-HT-selective 5,7-DHT lesions of the dorsal/medial raphé, suggesting action, relevant to aggression, at postsynaptic receptors. The 5,7-DHT lesions per se had no effect on either offensive or defensive behaviors. The failure to find changes in defense for either the 5,7-DHT lesions, or for eltoprazine given to intact or 5,7-DHT-lesioned rats, should be interpreted in light of the finding that defensive behaviors constituted only about 3% of the behaviors measured for the resident animals used in this situation. These results do agree with findings that male intruder defense against maternal attack in rats was unaffected by eltoprazine, or by fluprazine, a weak 5-HT<sub>1A/1B/2</sub> agonist (41). However, a number of recent studies suggest that eltoprazine may increase anxiety-like behaviors in both rats and mice (42–45) in situations other than those measuring response to conspecific attack, further suggesting caution in acceptance of the view that these 5-HT ligands have no effect on defensive behavior.

The 5-HT<sub>1A</sub> agonist, ipsapirone, has been reported to reduce defensive and flight behavior in defeated male rats (46).

#### *Mice*

In studies using both timid and aggressive isolate mice, buspirone slightly (circa 20%) but significantly reduced defensive postures in timid mice, and increased these in aggressive mice, but only at doses (10 and 20 mg/kg) associated with reduced locomotor activity. A lower dose (1 mg/kg) of buspirone without locomotor effects reliably reduced aggression in the aggressive animals but increased it in the timid mice (47). This pattern, of increased timid or defensive behavior in aggressive mice, but decreased defensiveness in timid mice or rats, has also been obtained with fluprazine (48–50). The common reports of opposite effects, on specific behavior categories, of drugs given to timid and aggressive isolates raises the question of possible differential neurochemical changes for these groups in response to isolation, additionally making it difficult to relate findings in such animals to those of unselected, nonisolates, particularly when a species difference (e.g. mouse–rat) is also involved (40).

A different paradigm involves isolated male mouse subjects as residents, evaluated in confrontations with conspecific intruders into their home cage. In a series of studies using this paradigm, Olivier et al. (41,51,52) found a clear reduction of escape and avoidance behavior with higher doses of the 5-HT<sub>1A</sub> agonists 8-OH-DPAT, ipsapirone and buspirone. 8-OH-DPAT also decreased defensive upright

postures at 0.25–6.25 mg/kg. In the same series, the serotonin reuptake blocker, fluvoxamine, increased defense (41) while tending to decrease (51) or (at lower doses) having no impact on (41) avoidance. Eltoprazine sharply increased defense in one set of tests (51) but not in a subsequent series (41), while increasing avoidance, at some doses, in both. Fluprazine produced a behavioral profile suggesting increased avoidance, but the effect was not reliable (51). The 5-HT<sub>1A/1B</sub> agonist, RU 24969 failed to influence defense but appeared to increase avoidance, whereas the 5-HT<sub>2C/1B/weak1A</sub> agonist, TFMPP, increased defense with no effect on avoidance (51). 5-Me-ODMT (5-HT<sub>1A/2C/1B</sub> agonist) increased defense at the highest dose used (10.0 mg/kg) but decreased avoidance (41). More recently, Bell and Hobson (53) examined the effects of several 5-HT<sub>1A</sub> ligands and 5-HT<sub>1B</sub> agonists on defensive reactions of mice in a resident–intruder paradigm. They demonstrated that the 5-HT<sub>1A</sub> agonists 8-OH-DPAT (0.25–1.25 mg/kg), ipsapirone (0.1–10.0 mg/kg) and MDL 73005EF (0.25–8.0 mg/kg) attenuated a score combining several defensive behaviors (i.e. evade, defensive upright, defensive sideways, submissive upright, frozen crouch). Although these effects were not associated with concomitant reduction in activity, the activity baselines of the control groups tended to be very low. This, plus the finding that all three drugs reduced offense at at least one of the doses reported, makes it difficult to determine how specific are the effects of these drugs on defensive behavior (53). Administration of the 5-HT<sub>1A</sub> antagonists pindobind 5-HT<sub>1A</sub> (0.5–10.0 mg/kg), SDZ 216–525 (0.025–1.0 mg/kg) and (+)-WAY-100135 (1.0–10.0 mg/kg) did not systematically change resident defensive responses (54–56). Finally, these authors showed that the mixed 5-HT<sub>1A/1B</sub> agonist CGS 12066B enhanced elements of defensive behaviors, whereas the more selective 5-HT<sub>1B</sub> agonist CP-94,253 failed to alter these responses (57).

While these profiles do not suggest any clear relationship for specific receptor subtypes and particular defensive behaviors they do indicate a rather consistent, though not necessarily high-magnitude, increase in some aspect of conspecific male defense to male attack for compounds sharing 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub> and/or 5-HT<sub>2C</sub> receptor affinity. In contrast, more selective 5-HT<sub>1A</sub> compounds tend to decrease avoidance, but involvement of sedation or motoric effects cannot be totally discarded. Finally, results obtained with CP-94 253 suggest that selective activation of 5-HT<sub>1B</sub> receptors has minimal impact on conspecific defense in mice.

#### *Vocalizations in a conspecific threat/attack context*

During conspecific threat/attack, both low frequency (18–32 kHz) and high frequency (> 30 kHz) ultrasounds, as well as sonic vocalizations, are made: the latter are most common in conjunction with the reception of a bite or other painful experience. Miczek et al. (58) have reviewed the effects of GABA<sub>A</sub> and 5-HT anxiolytics on these and other vocalizations. In male rats threatened by (protected) confrontation with a conspecific that had previously defeated them, diazepam (1–6 mg/kg) selectively reduced the high frequency ultrasounds, while gepirone (0.3–6 mg/kg) decreased only the low frequency USV. Neither drug reduced sonic vocalizations and these drugs did not affect ultrasounds or specific defensive behaviors during actual

attack on the subjects (59). Similarly, Tornatzky and Miczek (60) found that diazepam and gepirone reduced the high and low frequency (respectively) ultrasonic vocalizations of naive rats placed in the (empty) cage of a conspecific and also reduced the tachycardia and hyperthermia that occurred at this time. However, neither drug altered the audible or low frequency ultrasounds during subsequent agonistic confrontations with the resident. They suggest that anxiolytics may be more effective in reducing ultrasounds made in a (threat-) anticipatory context rather than during actual traumatizing events, a view that is in agreement with findings of a relatively specific effect of anxiolytics on risk assessment behaviors that occur specifically in the context of anticipated or potential threat (7).

High frequency ultrasounds are also emitted when conspecifics are encountered in situations in which the threat component is much less clear. When confronted with an anesthetized, same-sex conspecific in a neutral test cage, both male and female rats emitted only high frequency (> 35 kHz) ultrasonic vocalizations, with females making more cries than males (61). In females, but not males, the number of these calls was reduced by gepirone, (1.0–10.0-mg/kg) and by diazepam, at 3.0 mg/kg.

#### *Other relevant studies*

Since the dorsal raphe nucleus is the source of much of the serotonin available to forebrain areas, it is notable that, during conspecific agonistic encounters, the firing rate of dorsal raphe nucleus neurones increases for defensive tree shrews (*Tupaia belangeri*) engaged in agonistic conspecific encounters, with an even greater increase when an actual fight occurs (62). Some degree of specificity for this change is suggested in that firing rates decrease for the offensive partner in the same encounters.

#### LONGER-TERM DEFENSIVE REACTIONS TO CONSPECIFIC ATTACK: DOMINANCE AND SUBORDINATION

Rat, mouse, and primate groups or colonies have all been used for analysis of the long-term effects of agonistic interactions on both the successful and the defeated animal. A related paradigm involves longer-term measures taken on animals defeated in individual conspecific interactions. Systematically victorious animals tend to display "dominant-type" activities representing a pervasive style of interaction with same-sex conspecifics while strongly or systematically defeated animals display behavior patterns that have been characterized as "subordinate" or "submissive". The vast majority of such studies involve males, although some studies indicate that the physiological or behavioral mechanisms altered in chronically defeated animals may be different in males and females (63). Subordinates often display physiological changes such as higher levels of circulating glucocorticoids and are often regarded as providing a model of social stress (64). In addition to the physiological changes they show enhancement of defensive behaviors, and a general inhibition of nondefensive behaviors (e.g. (14,1)). Although such models present a number of problems in terms of analysis of drug effects (e.g. the time scale involved, and the potential mixture of conditioned and unconditioned effects), they may be particularly suitable for analysis of antidepressant action (e.g.

(64,65)). Much of the research utilizing such models has involved serotonergic mechanisms.

#### *5-HT effects on longer-term reactions to conspecific attack: dominance and subordination*

There is a great deal of evidence for some type of serotonin system involvement in the long-term consequences of conspecific defeat, and, indeed, in response to other chronic stressors. Woodall et al. (66) found that the selective 5-HT<sub>1A</sub> agonist 8-OH-DPAT (25 and 37.5 µg/kg) increased the rank order, evaluated by attainment of access to sweetened milk, of individual rats maintained in triads, without altering the animal's intake of sweetened milk or locomotor activity. Although the relationship between dominance based on access to food, and the behavioral and physiological changes seen during and after agonistic confrontation in rats is poorly understood, these results are consonant with those of Wilde and Vogel (67) that another 5-HT<sub>1A</sub> agonist, ipsapirone, (10 mg/kg, p.o.) ameliorates anxiety in unstable rat groups and reduces the enhanced voluntary ethanol intake of these animals. However, Korte et al. (68) reported that ipsapirone (5.0 mg/kg) produced a significant post-defeat increase in immobility, and further elevated corticosterone and catecholamine levels that had been increased following defeat, without altering these when measured prior to defeat. This finding of increases in both behavioral and physiological mechanisms associated with defense may reflect the differing outcomes of actions of ipsapirone (and other 5-HT<sub>1A</sub> receptor agonists) on pre- and post-synaptic receptor sites as influenced by parameters of drug administration. A single dose of the 5HT<sub>2</sub> antagonist amperozide has been reported (69) to reduce the social stress response when given to weanling pigs as they are first mixed in groups.

5-HT reuptake inhibitors and tricyclic antidepressants (TCA) both appear to reduce some of the long-term effects of social defeat. Because the TCAs also influence neurotransmitter systems other than serotonin, only a single such study will be reported here. Subordinate male tree shrews (*Tupaia belangeri*) show dramatic behavioral, physiological, and neuroendocrine changes when living in visual and olfactory contact with a dominant male conspecific (70). Daily oral administration of clomipramine (50 mg/kg), a tricyclic antidepressant that may work largely through changes in serotonin systems (e.g. (71)) counteracted both the behavioral and endocrine effects of subordination. Over time it produced a partial or complete normalization of subordination-induced changes in marking and grooming behavior, locomotor activity, and risk assessment, as well as urinary cortisol and norepinephrine excretion.

In visible burrow system (to be described in 6.1 below) colonies, daily injections of the 5-HT reuptake inhibitor fluoxetine (10 mg/kg s.c.) to subordinate male rats resulted in reversals of dominance in some colonies, with an increase in behaviors such as attempts to copulate in the presence of the dominant rat (72).

#### *Other studies*

Further evidence of the involvement of serotonin mechanisms in conspecific defense may be found in differences in regional levels of brain 5-HT and its major

metabolite, 5-HIAA for dominant and subordinate animals. Submissive mice have been reported to have elevated levels of 5-HIAA in hypothalamus, hippocampus and brainstem (73). Blanchard et al. (74) have found higher 5-HIAA levels for subordinate rats in amygdala, hippocampus, spinal cord and entorhinal cortex, with higher 5-HIAA/5-HT ratios in midbrain, spinal cord, and hypothalamus. Both studies thus agree in suggesting a high level of activity in 5-HT systems in subordinate males, with considerable overlap between the two studies in the particular areas involved. This relationship appears not to be confined to mammals: For example, subordinate male anolis lizards (*A. carolinensis*) show higher 5-HIAA/5-HT ratios after one hour of grouping with a dominant (75), declining thereafter, with other changes in monoamine systems as well. In bicolor damselfish (*Pomacentrus partitus*) 5-HIAA/5-HT ratios are elevated in telencephalon for attacking as well as the defending member of an interacting pair, compared to controls (76). These findings agree with those of a large number of studies indicating that various stressors increase serotonin metabolism, particularly in limbic forebrain structures (e.g. (77)).

Fontenot et al. (78) reported that only 5-HT and its major metabolite were altered (dopamine and norepinephrine and related metabolites were also examined) in the prefrontal cortex (PFC) of socially stressed adult male cynomolgus macaques (*Macaca fascicularis*). However, in contrast to the above studies, lower levels of both 5-HT and 5-HIAA were obtained in animals subjected to a period of social stress (group reorganization) that ended one to four months prior to sacrifice, compared to animals in stable groups which were treated as nonstressed controls. In addition, this previous stress group had lower PFC 5-HT concentrations than those of a recent stress group, which had been reorganized during the 14-month period immediately prior to the analysis. While the differences between these findings and those showing higher levels of 5-HT or higher 5-HIAA/5-HT ratios may reflect a number of species and procedural differences, they may also emphasize the importance of regional differences in the effects of social stress on serotonin systems. McKittrick et al. (79) reported reduced binding to 5-HT<sub>1A</sub> receptors for group-housed rats compared to controls in a number of sites in hippocampus and dentate gyrus, but increased binding to 5-HT<sub>2</sub> receptors in parietal cortex for subordinates compared to controls. Kudriavtseva et al. (80) found a number of regionally specific 5-HT system changes in defeated male mice, including the increased 5-HIAA/5-HT ratio in hippocampus. Again, some, but not all, 5-HT system changes were found in both victorious and defeated mice relative to controls, suggesting a differentiation between the stress of social interaction, and the (greater? different?) stress of defeat.

Serotonin depletion studies, generally tending to have a primary focus on offensive behavior or dominance, with a secondary emphasis on defense, have had mixed results with reference to both of these. Although depletion of forebrain 5-HT by intraventricular administration of 5,6-DHT (81) has been reported to increase dominance or offense, as has intrahypothalamic 5,7-DHT-induced serotonin depletion (82), Sijbesma et al. (40) found no change in offense following 5,7-DHT lesions of the dorsal/medial raphé. Only the last of these, as noted earlier, directly measured defense in the treated subjects, showing no effect. However,

File et al. reported (83) that more selective depletion of amygdaloid 5-HT reduces dominance and enhances submission in conspecific interactions.

#### DEFENSIVE ANALGESIA

##### *Opioid and nonopioid analgesia to conspecific attack*

Conspecific defense in rats (*Rattus norvegicus*) and mice (*Mus musculus*; *Peromyscus maniculatus*) is associated not only with characteristic defensive, submissive and escape behaviour, but also with a range of distinctive physiological changes. These include often profound alterations in endocrine, cardiovascular and neurotransmitter function. Several research groups (Kavaliers, Miczek, Rodgers, Siegfried; reviewed in (84,85)) have shown that exposure to conspecific attack (particularly in male mice) is also associated with major changes in reactivity to noxious stimulation. Intriguingly, while initial work in this field consistently demonstrated that exposure to attack results in the activation of a central opioid-mediated pain inhibitory system, further research revealed that the type of analgesia (opioid or nonopioid) observed depends critically upon the nature of the agonistic experience. Typically, opioid analgesia occurs in response to prolonged and/or intense conspecific attack, whereas nonopioid analgesia is evident during the initial stages of such encounters (e.g. upon initial defeat). Theoretically, it has been proposed (86) that these analgesic reactions subservise *context-specific* defensive functions, with the former facilitating a passive strategy (i.e. immobility) and the latter an active strategy (i.e. fight or flight). Furthermore, as nonopioid analgesia can even be elicited by the territorial scent of an aggressive conspecific, it has been interpreted as an anticipatory defense reaction linked to anxiety and, as such, subjected to detailed pharmacological investigation.

##### *BZS and nonopioid analgesia*

Consistent with their inhibitory effects on active forms of defense (i.e. defensive upright and escape), BZs have been found to inhibit non-opioid analgesia in defeated male mice. Furthermore, as this form of adaptive pain inhibition is not only blocked by low doses of BZ receptor agonists (e.g. diazepam, clonazepam, alprazolam) but also by BZ receptor antagonists (e.g. flumazenil) and inverse agonists (e.g. Ro15-3505), possible mediation by an endogenous BZ receptor inverse agonist has been proposed (87,88,85,89,90). However, other findings indicate greater complexity in the role played by BZ receptor mechanisms in this form of defensive analgesia. Thus, while a lack of effect of BZ receptor partial agonists (e.g. ZK 91296, CGS 9896) may be attributed to the weaker intrinsic efficacy of these compounds, neither efficacy nor potency considerations can easily account for the failure of certain BZ receptor full agonists (e.g. chlordiazepoxide, midazolam and ZK 93423) to block the response (87–89). Furthermore, although data obtained with agents such as Ro5-4884, PK 11195 and Ro5-5115 point to a role for non-neuronal BZ recognition sites in the mediation of nonopioid analgesia (89), the overall pharmacological profile obtained precludes any firm conclusions regarding the relative importance of neuronal and non-neuronal sites.

### 5-HT receptor ligands and non-opioid analgesia

In contrast to the somewhat variable effects observed with BZ receptor ligands, non-opioid analgesia in defeated male mice is potently and consistently inhibited by 5-HT receptor manipulations. In this context, several studies have shown that the response is completely blocked by low doses of 5-HT<sub>1A</sub> receptor agonists (8-OH-DPAT, buspirone, ipsapirone, gepirone, MDL 73005EF (91–93). Furthermore, these effects are stereospecific (94) and can be antagonized by (–) pindolol (a 5-HT<sub>1A</sub> antagonist) at doses which, per se, do not affect the basic response (92). Significantly, while defeat analgesia is unaffected by compounds which show high affinity for 5-HT<sub>1B</sub> or 5-HT<sub>2</sub> receptors (93), it is potently inhibited by a range of 5-HT<sub>3</sub> receptor antagonists (e.g. ondansetron, ICS 205-930, MDL 72 222, MDL 73 147; (95,96)). However, it is important to note that, in contrast to the complete inhibition observed with 5-HT<sub>1A</sub> receptor agonists, the 5-HT<sub>3</sub> antagonists invariably (albeit extremely potently) produce profiles of partial inhibition with at least some evidence for a peripheral site of action. Thus, while clearly confirming an important role for both 5-HT receptor sub-types in nonopioid defensive analgesia, the data suggest that 5-HT<sub>1A</sub> sites may be more critically involved.

### Defensive analgesia to predator exposure

Consistent with the proposed defensive function of environmentally-induced analgesia (for review (84), exposure to predators has been reported to reduce pain responsivity in various species. A classical example of this phenomenon is found in the writings of the Scottish missionary and explorer, David Livingstone who, during an expedition to the headwaters of the Nile, was attacked by a lion. Despite being shaken by the lion, much as a terrier shakes a rat, he reported no sense of pain but rather a sort of stupor or dreaminess similar to that experienced under chloroform (97). Parallel findings have more recently been reported in laboratory studies involving non-contact exposure of rodents to natural predators.

In the first such study, Lester and Fanselow (98) found that fifteen min exposure to a cat housed in an adjacent compartment produced a profound opioid-mediated analgesia in laboratory rats. These findings were subsequently confirmed by Lichtman and Fanselow (99), and extended to other species by Kavaliers and colleagues using both laboratory and feral subjects. In wild white-footed mice (*Peromyscus leucopus*), non-visual exposure to a natural predator (short-tailed weasel, *Mustela erminea*) elicited analgesia, the strength, time-course and mediation of which varied as a function of exposure duration (100). Thus, very brief (30 s) exposure produced a short-lasting analgesia that was insensitive to the opiate receptor antagonist naloxone (1 mg/kg) but which was blocked by 10 mg/kg flumazenil and 4 mg/kg diazepam; intermediate exposure (5 min) produced a longer-lasting analgesia that was sensitive to both opioid and benzodiazepine receptor ligands; prolonged exposure (15 min) resulted in a long duration analgesia that was sensitive to naloxone but not to either benzodiazepine receptor ligand. Essentially identical results were subsequently obtained in a related species, the deer mouse (*Peromyscus maniculatus*), with the interesting additional observation of population differences in response

patterns (101). Although a weasel-sympatric mainland sample of deer mice showed both forms of pain inhibition (short-lasting benzodiazepine-sensitive and long-lasting opioid-mediated), samples derived from a weasel-free island location showed only the opioid-mediated reaction. In a series of follow-up studies, employing weasel odor, Kavaliers et al. (102) and Kavaliers and Colwell (103,104) found that prolonged (15 min) exposure to the predator cue produced an analgesia that was blocked by naloxone (1.0 mg/kg) but not the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT (0.5 mg/kg) whereas brief exposure (30 s) produced an analgesia that showed an opposite pharmacological response (blocked by 8-OH-DPAT but not naloxone): sex differences were also evident in this study, with males showing a greater opioid response and females showing a greater non-opioid response. Very similar patterns of results were also obtained in laboratory mice (*Mus musculus domesticus*) exposed to the presence of an experienced predatory cat (105) and in juvenile meadow voles (*Microtus pennsylvanicus*) exposed to a garter snake (*Thamnophis sirtalis*), a major predator of the young of this species (106).

These data not only confirm the existence of multiple, temporally-organized, defensive analgesia systems in several mammalian species but also show remarkable correspondence with the outcome of research on pain inhibition in response to conspecific attack. Both lines of investigation support the view that short duration exposure to threat (aggressive conspecific or predator) produces a relatively short-lasting pain inhibition that is sensitive to benzodiazepine and 5-HT<sub>1A</sub> receptor ligands known to impact anxiety while prolonged exposure to the same stimuli results in an enduring opioid-mediated pain inhibition. As proposed by Rodgers (84), the former may function to facilitate active defenses (such as flight and fight) while the latter may facilitate passive defenses (freezing/immobility)—a view entirely consistent with the temporal organization of these defensive behaviour patterns.

### ANTIPREDATOR DEFENSE

Antipredator defensive behaviors vary systematically with features of the (predator) threat stimulus and situation, including the type of predator-related stimulus; distance between the subject and the threatening predator; whether the predator is present or signalled but absent; and as a function of time following confrontation with the predator (7,14,1,107,108). Because of the complexities of these relationships, analysis of drug effects on antipredator defense depends on the creation and use of situations that permit the elicitation and measurement of specific behaviors, in order to determine how these are modulated by drugs.

### Immediate reactions to a predator

The immediate defensive behaviors of most mammals to confrontation with a predator include flight (followed by avoidance), freezing, and defensive threat (including sonic vocalizations) and attack. Specific postures (e.g. the upright posture) may occur but are less systematic than are those seen to conspecific attack. For rats (wild *R. norvegicus*) and mice (Swiss-Webster) these behaviors

may be systematically and specifically elicited in a Fear/Defense Test Battery (F/DTB; e.g. (109)) for rats and a Mouse Defense Test Battery (MDTB; e.g. (110)). In these tests, the subject is placed in a long oval runway, permitting limitless flight, and approached by a predator, either a researcher (for the rat test) or a deeply anesthetized rat, hand-held and moved around the apparatus (for the mouse test). Systematic manipulation of predator movements; of situational characteristics (e.g. trapping the animal); and of subject-predator distance are used to elicit and measure specific behaviors.

In addition, a number of studies using other species or other paradigms have measured these or related behaviors in the context of confrontation with a predator.

#### *The extended or longer-term pattern of defense to a predator*

##### *The visible burrow system (VBS)*

The VBS, a habitat with a "surface" area in which food and water are found, plus tunnels and chambers designed to resemble actual burrows, was created specifically to enable rat or mouse subjects to demonstrate as full a range as possible of defensive reactions. Presentation of a non-attacking cat for fifteen minutes in the surface area, to mixed-sex rat groups, produced immediate flight to the burrow system, and freezing there for several hours (14). Other long-term reactions, seen after cat removal as well as in its presence, include: Antipredator Ultrasounds, circa 22 kHz ultrasounds emitted only by rats in the burrows while the cat was present and for about 30 min. after its removal (15), that may serve as alarm cries; Inhibition of nondefensive behaviors such as sexual and aggressive behaviors and eating and drinking, up to seven hours after cat presentation (14,1); and risk assessment (RA) activities oriented toward potential threat (in this case the open area where the cat was encountered) (7).

Because of results from tests involving drug manipulations, RA activities have come to be a particular feature of defensive behavior analysis in some laboratories (7). The defining characteristic of RA is orientation and attention to potential threat stimuli, which can be manifested in a number of ways, depending on the situation. In the VBS, active RA activities such as poking the head out into the open area and scanning reliably followed cat exposure, but only after a time lag of 4–7 h after cat exposure. Locomotion associated with RA typically involved flattening of the back and stretching of the animal's body in a stretched attention posture, described by Van der Poel (111) as an ambivalent behavior, reflecting both approach and avoidance tendencies. Pinel and Mana (112) have demonstrated that RA activities are associated with gathering of information concerning potential threat, and it is the feedback from these activities that is assumed to produce a characteristic decrease in defensiveness over time (7,1). This interpretation is supported by recent results from Williams et al. (113), indicating that twelve hours of exposure to cat odor, alone, facilitates the transition from freezing to active RA during a subsequent cat odor presentation.

##### *The anxiety/defense test battery*

The VBS itself is difficult to use with pharmacological

manipulations, because of the time required for transitions among behaviors. An Anxiety/Defense Test Battery (A/DTB; (114,115)) consists of three tests providing simplified measures of several behaviors, in particular those associated with the longer-term behaviors involved in the transition from immediate antipredator reactions to normal, nondefensive, behavior. These three tests provide several measures of RA, and inhibition of behaviors such as eating or drinking after cat or cat-odor presentation. Freezing, locomotion, grooming, rearing, etc. are measured in the same tests, in part to provide evidence of possible nonspecific or sedative effects of drugs.

#### BZ EFFECTS ON IMMEDIATE OR LONGER-TERM DEFENSIVE REACTIONS TO A PREDATOR

The introduction of BZs to clinical practice in the early 1960s was accompanied by extensive publicity concerning their ability to tame captive animals of diverse species. As the majority of these studies were based upon the assessment of reactivity to human approach/handling, they may be collectively considered as precursors to more objective recent analyses of the effects of these agents on the defensive repertoire. Examples include reported reductions in defensive responses (e.g. biting) to human intrusion in cynomolgus monkeys, squirrel monkeys, baboons, stump-tail macaques, marmosets, asses, dingo, fallow deer, lynx and sea lions (116–119). Studies specifically focusing on particular antipredator defensive behaviors and measuring these in conjunction with BZs include the following.

##### *Wild rats: the F/DTB*

Three BZ full agonists have been tested in the F/DTB using wild rats as subjects (120). The effects of diazepam, chlordiazepoxide and midazolam were remarkably consistent, with essentially no effect on flight, avoidance, or freezing (with the exception of a possibly sedation-related high-dose midazolam reduction in flight speed). Although not reliable, a clear trend toward reduced defensive biting attack was evident for each compound. However, each of these compounds reduced (sonic) defensive threat vocalizations in one or more of the three situations tested.

##### *Laboratory rats: the A/DTB*

In the A/DTB, diazepam (2 and 4 mg/kg, i.p.) and chlordiazepoxide (5 and 10 mg/kg) both reliably reduced proxemic avoidance of the threat stimulus, thus increasing RA (114). Both drugs also reduced the threat-induced inhibition of eating and/or drinking. At these dose levels, there was no drug effect on freezing, although chlordiazepoxide at sedative doses reduced freezing. Diazepam and chlordiazepoxide *increased* RA when it was measured against a baseline of freezing and proxemic avoidance in a test involving presentation/removal of a cat (114), but diazepam *decreased* RA (stretch attend/approach) in the cat odor test in which vehicle controls showed a behavioral baseline of about 30% freezing with high levels of RA (115). The reduction in RA in the cat odor test with 2.0 mg/kg diazepam has recently been replicated by Anderson and Taukulis (in preparation). These findings suggest that the defense baseline (i.e. the level of specific defensive behaviors shown by controls in a given situation) is an extremely important factor in the effect of diazepam or



chlordiazepoxide on RA activities in the rat. These behaviors increase when drug is given to animals that are freezing and showing proxemic avoidance to high level threat, but decrease when the drug effect is measured against a baseline that includes relatively high RA, in both cases mimicking the effects seen as defensive behavior declines and returns to normal over time.

#### Mice

Results obtained with five BZ receptor full (i.e. alprazolam, clonazepam, clorazepate, chlordiazepoxide and diazepam) and three partial agonists (bretazenil, imidazenil and Ro 19-8022) showed that these compounds generally reduce defensive threat/attack reactions induced by physical contact with the threat stimulus in the MDTB (110,121,122), without systematically altering flight (see below) or freezing. In addition, the MDTB, unlike the FDTB, elicits RA in mice during confrontation with the predator and all of these compounds also reduced RA activities observed when the mouse was chased by the rat. This reduction in RA by BZ agonists was specific to the chase situation, and did not occur when subjects were trapped in the runway and confronted by the rat. All full agonists and the partial agonist Ro 19-8022 also counteracted an increase in situational escape attempts by the mouse after the predator had been removed from the test area. Finally, only the two high-potency BZs, alprazolam (after chronic administration only) and clonazepam, and the partial agonist Ro 19-8022 reduced flight reactions at non-sedative dose levels. Overall, these behavioral profiles indicate that high-potency BZ receptor full agonists affect a wider range of defensive behaviors than classical BZs (e.g. chlordiazepoxide, clorazepate and diazepam). These latter, in addition to showing a more selective profile of effects, were superior in reducing defensive responses in comparison to partial agonists with weak intrinsic activity like bretazenil and imidazenil.

#### Cats

Langfeldt and Ursin (123) reported that diazepam (1.0 mg/kg) reduced a composite defensive threat/attack score, but failed to alter a flight/startle/withdrawal composite, in response to human approach and manipulation, in wild-trapped feral cats.

#### Primates

Scheckel and Boff (119) reported that biting in cynomolgus monkeys (*Macaca philippensis*) was reduced by each of a number of 1,4-BZs, including diazepam (at 1.0 mg/kg p.o.) and chlordiazepoxide (1.0 mg/kg). In squirrel monkeys (*Saimiri sciureus*) diazepam and chlordiazepoxide also reduced defensive biting at similar doses, lower than those required to change avoidance responding. Diazepam (3.0 mg/kg) also decreased the duration, but not the intensity, of defensive threat vocalizations of the squirrel monkeys. Vellucci et al. (124), working with groups of talapoin monkeys (*Miopithecus talapoin*), have reported increased visual monitoring (increased RA?) following administration of the BZ inverse agonist,  $\beta$ -CCE (375  $\mu$ g/kg, IM), to subordinate colony males.

In a primate model (108) in which young rhesus monkeys, separated from their mothers, are confronted by an experimenter, displaying or not displaying a menacing

stare at the subject, two vocalizations are emitted. These, "coo" and "bark", responded differently to 1 mg/kg diazepam, the former unchanged and the latter strikingly and significantly reduced. Given the interpretation that the latter, only, represents a defensive threat vocalization, these data agree directly with the reduction in wild rat threat vocalization found with each of three BZs. However, freezing and crouching were also reduced reliably by diazepam in the infant Rhesus monkeys, which was not obtained in the wild rat studies.

#### Summary

The effects of BZ compounds on immediate and longer-term reactions to the presence of or contact by a predator are thus remarkably consistent. Studies in rats, mice, cats, and a variety of primates indicate that BZ receptor agonists produce reductions in defensive threat/attack (including defensive vocalizations) and characteristic changes in RA (i.e. a decrease in RA against a strong RA baseline, but, in rats, increased RA when evaluated against a freezing baseline). Some mouse studies also showed an effect on attempts to escape the situation in which the predator was presented. Studies measuring flight/avoidance/freezing in rats, and cats provided no clear evidence of change, although freezing did decline in infant monkeys. In mice, two high potency BZs, alprazolam and clonazepam, reduced flight as well. Notably, these are different than other BZs commonly used in clinical settings in that they reduce panic as well as anxiety (e.g. (125)), a behavior change seen also with non BZ panicolytic drugs.

#### 5-HT EFFECTS ON IMMEDIATE AND LONGER-TERM REACTIONS TO A PREDATOR

##### Wild rats

In wild rats in the Fear/Defense Test Battery, two 5-HT<sub>1A</sub> agonists, buspirone and gepirone (5.0–20.0 mg/kg for each) generally failed to alter avoidance or freezing, but increased the number of subjects that could be approached by the experimenter to the point of pick-up, and reduced a number of defensive behaviors associated with such approach; boxing, biting, jump-attack (120). Gepirone effects were more clearly dose-dependent. Both, but especially gepirone, reduced defensive threat and attack to stimuli such as vibrissae stimulation, dorsal contact, or an anesthetized conspecific. Jump/flinch reactions to back-tap were also reduced. It is noteworthy that the three BZs (diazepam, chlordiazepoxide and midazolam) and the two 5-HT<sub>1A</sub> agonists used in the F/DTB all showed similar changes in defensive vocalization, but that gepirone and buspirone reduced a number of additional defensive behaviors.

##### Laboratory rats

The A/DTB. As in the F/DTB, 8-OH-DPAT produced a range of effects in the A/DTB (126), decreasing avoidance, freezing, and grooming, and increasing transits to the stimulus area (an RA measure). It also reduced the threat-induced inhibition of eating but not drinking. Shepherd and Rodgers' (127) report that 8-OH-DPAT enhances feeding in mice in the presence of an aggressive attacking conspecific adds to the view that 5-HT<sub>1A</sub> agonists particularly enhance eating in extremely stressful situations. These 8-OH-DPAT

effects in the A/DTB were more pronounced in females; gender differences and sex  $\times$  drug interactions are a common feature of the A/DTB (128).

Gepirone (5.0 and 10.0 mg/kg) used in an earlier and slightly different version of the A/DTB, (unpublished findings) also decreased proxemic avoidance and freezing, and increased eating, drinking and transits in situations associated with a cat. Other effects were obtained with the higher dose, and sedative effects may have been involved. Buspirone (1.0–10.0 mg/kg) produced anxiolytic-like effects on RA at all doses. However, only the lowest dose was associated with reduced avoidance of the cat odor stimulus, suggesting that the anxiolytic effects of this compound are limited to a narrow dose range and diminish above 1.0 mg/kg (Anderson and Taukulis, unpublished findings). The dose–response differences between gepirone and buspirone, both 5-HT<sub>1A</sub> agonists, are compatible with the view that buspirone also increases firing of catecholaminergic neurones (129), with these increases perhaps effectively competing with serotonergic changes at higher doses.

An additional study with the non-selective 5-HT<sub>2</sub> receptor antagonist ritanserin failed to demonstrate convincing influence of the drug on antipredator defense in the A/DTB (130). Finally, in the A/DTB as well as in the F/DTB, the 5-HT<sub>3</sub> antagonist ondansetron was devoid of effects on all defensive behaviors measured (131).

### Mice

The two 5-HT<sub>1A</sub> agonists (8-OH-DPAT, 0.05–10.0 mg/kg, and gepirone, 2.5–10.0 mg/kg) tested in the MDTB presented a similar profile to that of the classic BZs (ie chlordiazepoxide, diazepam and clorazepate), reducing defensive threat/attack responses and situational escape attempts but failing to affect flight (132). Although both of the 5-HT<sub>1A</sub> agonists, like the classic BZs, decreased RA, this decrease was obtained in a different subtest of the MDTB than that in which classic BZs were effective. The effects of several selective (pirenperone, MDL 100,907, SB 206553) and non-selective (mianserin) 5-HT<sub>2</sub> antagonists have also been investigated in the MDTB (132,133). Unlike BZs and 5-HT<sub>1A</sub> agonists, these 5-HT<sub>2</sub> agents only weakly and/or non-specifically affected defensive behaviors. As an example, the 5-HT<sub>2B/2C</sub> antagonist SB 206553 significantly decreased defensive threat and attack responses at doses that also suppressed locomotor activity. Similarly, the preferential 5-HT<sub>2A</sub> antagonist pirenperone decreased several defense reactions including flight and escape attempts at doses also impairing motor responses. Finally, the selective 5-HT<sub>2A</sub> antagonist MDL 100,907 weakly, albeit significantly, reduced one RA (i.e. number of stops) measure. Taken together with the negative findings obtained with the non-selective 5-HT<sub>2</sub> antagonist ritanserin in the rat defense test battery, these results for mice suggest that antipredator defense may not primarily involve central 5-HT<sub>2</sub> receptor subtypes. By contrast, data obtained with the 5-HT<sub>1A</sub> agonists in these test batteries strongly suggest that this receptor may be involved, although perhaps not as strongly or selectively as GABA/BZ receptors, in the modulation of antipredator defense responses in rodents.

### Primates

Defensive responding (“defensive unrest”) in marmosets

and cynomolgus monkeys, provoked by human approach/threat, is potently inhibited by 5-HT<sub>1A</sub> agonists (e.g. buspirone) and 5-HT<sub>3</sub> antagonists (e.g. ondansetron) (134,46). However, interpretation of the latter findings is uncertain in view of the limited behavioral analyses employed.

### Predator exposure effects on 5-HT systems

In addition to studies measuring effects of manipulation of 5-HT systems on response to a predator, there are a few studies measuring effects of predator exposure on 5-HT systems. Wallatschek and Raab (62) reported dramatic (+187%) increases in the firing rate of dorsal raphe nucleus neurones in tree shrews in response to approach and insertion of the experimenter’s hand into the subject’s nest box. As noted earlier, dorsal raphe firing rates also increased in the tree shrews to conspecific attack. In contrast to the tree shrew findings, however, unit activity in the cat dorsal raphe nucleus was unaffected by the presence of a barking dog (135). Rueter and Jacobs (136), using microdialysis techniques, examined serotonin release in the rat forebrain induced by fifteen min. noncontacting exposure of rat subjects to a cat. Areas showing increases in 5-HT release included hippocampus, amygdala, striatum and prefrontal cortex. Enhanced 5-HT release was not specific to cat exposure but also seen in response to tailpinch, swim and environmental events, suggesting a relationship to alertness/activity rather than to defensiveness per se. Given the considerably higher defensiveness to human approach and handling manifested by wild, compared to laboratory rats, findings suggesting consistent differences of serotonin systems in median raphe, dentate gyrus, and entorhinal cortex between the strains in laboratory settings where human contact is frequent might also reflect enhanced responsivity to such contact in the wild animals (137).

### PROBLEMS ASSOCIATED WITH THE USE OF THESE MODELS IN PRECLINICAL PSYCHOPHARMACOLOGY STUDIES

Many of the problems associated with the study of defensive behavior arise from the extreme sensitivity of defensive behaviors to relevant features of the threat stimulus and situation, such as threat-to-subject distance and particular threat stimulus movements. The problem is differentially represented in conspecific-attack models, and in antipredator models, because the former typically involve an attacking conspecific that is relatively unconstrained, while the latter almost always involves the use of a predator or predator feature that enables considerable control over the actions of this stimulus. This difference has been responsible for not only the greater variability in results of pharmacological studies using conspecific-attack models, but has also determined some of the specific measures used in studies involving the two paradigms.

### Problems with conspecific-attack models

The core problem in conspecific-attack models is the attacking conspecific. First, there is the problem of variation in the behavior of the attacker, from one attacker to the next, or even from one session to the next, for the same attacker. The use of highly experienced and maximally attacking, or

alternatively, of nonattacking, rats may provide ways of minimizing both types of variability. However, the latter choice is likely to produce an inadequate threat stimulus except in particularly timid subjects, introducing the problem of subject selection and possibly reduced generalizability of results, or in those that have had previous experience of attack. Attack, present or previous, introduces pain as a factor in responsivity, and previous attack also brings learning, and memory systems into the paradigm. In such cases the conspecific defense paradigms share something of the problems of more traditional aversive learning models in that they appear to *require* some involvement of all of these systems, greatly adding to the analytic complexity of the situation. Although learning or memory factors may also be involved in antipredator paradigms, somatic pain can be discounted as a mechanism in defensiveness, as can the necessity for any previous contact with such stimuli. This suggests a stronger role for learning in the defensiveness of laboratory rodents to attacking conspecifics than in reactions to a predator, a view supported by comparisons of reactions to alpha odors (113) as opposed to those of predators (138).

As discussed earlier, a particularly difficult problem with the use of conspecific attack models in analysis of pharmacological effects is that attacker behavior may be very sensitive to defender drug state (the converse is also true: undrugged defenders may show significant and sometimes surprising changes in behavior when attacked by drugged residents, e.g. (53)); presenting an alternative avenue for drug effects on defender behavior, through an alteration of attack. This mechanism is often ignored as a possibly confounding factor in studies of conspecific defense, although the magnitude of the problem can be at least roughly estimated through analysis of attacker behavior as a function of defender drug treatment, for example in the Piret et al. (24) study discussed earlier. It is notable that, when studies do report the behaviors of attackers toward drugged defenders, these are often seen to change; moreover the changes seen may not always reflect drug-induced behavior change in the defender (e.g. (25)).

A final analytic problem with conspecific attack is that it consists of a series of events in which specific attacker behaviors respond rapidly to sometimes subtle movements of the defender. These relationships have been analyzed and described in detail in a number of recent articles by Sergio Pellis (e.g. (139)). Thus even if attacker behavior could be standardized in terms of a constant intensity, both the rapidity of conspecific attack and its responsivity to individual movements of the defender increase the difficulty of using conspecific attack behaviors as a series of standard stimuli to elicit specific defensive behaviors in order to measure the effects of pharmacological agents on these behaviors.

#### *Problems with antipredator models*

Antipredator models do not escape the difficulties arising from the extreme sensitivity of defensive behaviors to relevant features of the threat stimulus and situation. However, these problems are somewhat easier to control with antipredator paradigms, given that i) humans can serve as predators for a considerable range of subject species, such that a detailed script of "predator" action can be followed;

ii) terminally anesthetized individuals of predator species can elicit a wide range of defensive behaviors if moved appropriately, providing another avenue for the production of finely controlled and timed movements; iii) partial predator stimuli (e.g. cat odor) also elicit defense-related responses; while these may elicit more intense responses if associated with prior experience with the actual predator, no prior experience is required for some level of defensive responding.

None of these solutions is perfect. While tactic i) works well with subjects that are highly defensive to humans, most (domesticated) laboratory animals are not. Thus wild rats have been used for some of the basic studies of the organization of defensive behaviors. This may well be justifiable on the basis that animals defensive to humans probably provide more representative examples of basic mammalian neurobiological defense systems than those that have been domesticated. However, domesticated laboratory rat strains are frequently used in studies involving exposure to a non-human predator, as well as to painful threat stimuli, including attacking conspecifics, producing a strain or at least subject selection difference in comparisons across these paradigms; a difference that might have a significant impact on the findings of relevant drug studies. Tactics ii) and (especially) iii) may provide suboptimum threat stimuli. In addition, the possible role of the subject's drug status on predator behavior has not been investigated and could prove to represent a potentially potent phenomenon. However, since in these animal models the predators typically either do very little (e.g. A/DTB; sit quietly until removed from the situation) or, follow a rehearsed script (e.g. Kalin primate model: F/DTB) it does not seem likely that this is an important source of variability in these antipredator paradigms.

Although the manipulations required to achieve great control over the movements of the threat stimulus in antipredator paradigms may serve to reduce the overall potency of this stimulus compared to that of predators in the real world, the analytic power attained by the use of such highly controlled stimuli is immense, permitting the repeated and selective elicitation of a specific defensive behavior (e.g. defensive threat and attack). Used appropriately, in situations in which other defensive behaviors are also elicited and measured, this permits an evaluation of the effects of drugs or other manipulations on individual components of the defense pattern that is both more sensitive (re. individual behaviors) and, as shown in this review, yielding of more consistent results than does conspecific attack as a threat stimulus.

#### *Conspecific-attack and antipredator paradigms as chronic stressors*

Both conspecific-attack and predators may serve as severe, long-term stressors. Conspecific situations have been much more commonly used in this capacity, providing studies of chronic social defensiveness in subjects of a variety of species. Antipredator situations over equivalent lengths of time are rare, perhaps because they seem likely to produce either a considerable degree of habituation, or, consumption of the subject by the threat stimulus. The latter, of course, can be prevented by a barrier between the two, and some recent studies (e.g. (140)) suggest that even such a

barrier fails to produce rapid habituation of defensiveness of laboratory rats to a cat, suggesting that chronic antipredator stress models may be more suitable than previously believed.

Nonetheless conspecific or social stress situations do have the considerable advantage of providing both a dominant and a subordinate animal for comparison with controls (choice of an appropriate control group is another thorny issue but outside the scope of this treatment). This is useful because, while subordinates are typically highly stressed animals, dominants of laboratory (64), and wild animal groups (141) may also be stressed. As data on the changes associated with dominant and subordinate status emerge, it appears that the difference between the two is not merely quantitative, but may involve different patterns of effects in both brain/peripheral neurochemical systems, and behaviors. These differences, potentially relevant to a variety of defense-related psychopathologies, could not be analyzed in an antipredator model.

Conspecific situations, however, are inherently confounded for the study of gender effects in defense, or of the impact of pharmacological manipulations on these differences. Gender differences certainly do occur in a conspecific defense situation but they are virtually uninterpretable in that context because of the high magnitude difference in attack by rats, mice, and most other mammals on conspecific males as opposed to females. In the context of responsiveness to predator presentation, gender differences are striking, and potentially permit insights into hormone-neurotransmitter interactions (128); this also provides an additional rationale for the development of chronic antipredator models.

#### *Problems with the behavioral baseline*

An additional set of problems in analysis of pharmacological effects in defense may reflect, not the particular paradigms used, but the defense process itself. Defense is not one behavior but many, and they cannot and do not all occur at the same time. Since most test situations used to evaluate defense elicit a very high magnitude response, competition among defensive behaviors is problematic.

The problem is perhaps most acute with RA, since in rats the more active forms of RA do not occur until freezing and proxemic avoidance decline—reflecting a diminution in the overall magnitude of defensiveness—to a level permitting some approach to the stimulus. Thus a decline in defensiveness for a subject that is freezing will increase RA. However, a decline in defensiveness for a subject that is actively risk assessing will involve a reduction in RA, often along with an increase in the nondefensive behaviors that are suppressed during active defensive responding. It should be emphasized that this view is not a theoretical one, but is based on detailed analysis of the time course of defensive behavior following a single, powerful, aversive event, exposure to a predator (14,1). This process is consonant as well with the results of studies indicating that exposure to threat stimuli provides information about the dangerousness of these stimuli (112) such that when no danger is present this information will lead to a further decline in the magnitude of defensiveness.

The outcome is that in species showing this pattern, defense-reducing manipulations will have different effects

on RA, depending on the initial baseline for this *and other* behaviors. Although anxiolytic drugs would be expected to increase RA when freezing and proxemic avoidance are high and RA low, and to reduce RA when it is high, this is not simply a “rate-dependent” process. Certainly increased RA would not be the result if anxiolytics were given when RA was low in a nonthreatening situation. Instead, the differential effect of anxiolytics in the two situations appears to reflect the defense process, as it involves behaviors which, in rats, appear (in an active form, at least) only at intermediate levels of defensiveness. Since it should always be possible to obtain a behavioral baseline for controls in relevant situations this should not be an insurmountable problem in analysis. However, this may be an important factor in some of the already obtained inconsistent results, which are by no means rare, in studies of anxiolytic drugs applied to animal models of anxiety that incorporate some elements of the defense pattern. Fortunately for ease of analysis, not all species show this complex pattern. In particular, laboratory mice appear to display RA behaviors freely, as part of their high level defensive response to intense threat stimuli. While this particular pattern may be disadvantageous for the individual mouse, it considerably simplifies analysis of drug effects on RA. However, as noted above, effects of a wide range of drugs on RA in rats and in mice appear to be quite congruent, given the differences in baseline for this activity, promoting the belief that such activities do respond selectively to anxiolytic drugs, across different test species.

Although these factors produce complications in evaluation of the effects of drugs on RA, the necessity to make such evaluations reflects more than the possibility that RA is a marker for changes in the level of defensiveness. Given the joint role of RA in either identifying and localizing threat stimuli, or determining that the stimulus is absent/nonthreatening, one mechanism for the action of anxiolytics on reduction of anxiety may be through RAs effect on learning of stimuli associated with aversive events. This concept suggests that RA, in situations in which it occurs in normal subjects, is an integral component of the process of learning or extinguishing associations between neutral stimuli or situations and unconditioned threat stimuli. Thus a reduction in RA in an aversive conditioning situation would be expected to produce deficits in the acquisition of conditioned aversive responses, leading to disruptions in emotion-linked memory of aversive events. However, when an unconditioned or previously conditioned aversive stimulus alone is presented, RA reductions may impair the process of determining that the stimulus is not threatening, leading to deficits in extinction.

#### ADVANTAGES OF USE OF THESE MODELS IN PRECLINICAL PSYCHOPHARMACOLOGY STUDIES

To set against the disadvantages in the use of such models including, in addition to the above, the obvious fact that they tend to be (but are not necessarily) more time and labor intensive than standard drug tests, is the single substantial advantage: Used with adequate knowledge of the behavioral systems involved, they are capable of providing much more detailed and specific information about drug effects on defensive behaviors than are other tests. Although the reviewed results of conspecific defense tests do indicate

an undesirable level of variability, due in part to inherent problems with the use of conspecific attackers, it should be noted that much of this literature is older, and does not involve some of the possible solutions (e.g. standard non-attacking opponents; barriers between the subject and the attacker) to these problems. The problems of antipredator models are less pervasive, as is reflected in the much more consistent outcomes of these procedures. Finally, although a great deal of work is necessary in the development of such "naturalistic" or "ethological" models of defense, individual tests using these procedures do not need to repeat this developmental work. Often, the drug test paradigms

compare favorably with standard procedures (e.g. conflict models) in terms of the time and effort required to obtain a result. Moreover, the result obtained is one that the standard procedure could never produce, regardless of the time and effort involved.

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