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Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion

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Abstract

The advantages of using predator-related odor stimuli to study emotional responses in laboratory tests depend on whether such stimuli do elicit a relatively complete pattern of emotionality. This has been confirmed for cat fur/skin odor stimuli, which elicit a range of defensive behaviors in rats that may be reduced by anxiolytic drugs, produce residual anxiety-like behavior in the elevated plus maze and support rapid aversive conditioning to the context in which they were encountered. Although the synthetic fox fecal odor, trimethylthiazoline (TMT), elicits avoidance similar to that seen in response to cat fur/skin odor, this avoidance does not respond to anxiolytic drugs. In addition, TMT does not produce residual anxiety-like behaviors in the elevated plus maze, nor does it support conditioning.

As natural cat feces also elicit avoidance but fail to support conditioning, it is possible that the ability of a predator-related odor to serve as an effective unconditioned stimulus (US) relates to its predictive status with reference to the actual presence of the predator. Avoidance per se may reflect that a stimulus is aversive but not necessarily capable of eliciting an emotional response. This view is consonant with findings in a Mouse Defense Test Battery (MDTB) measuring a wide range of defensive responses to predator exposure. A contextual defense measure that may reflect either conditioned or residual but unconditioned emotional responses was almost never reduced by drug effects unless these also reduced risk assessment or defensive threat/attack measures. However, reductions in contextual defense without changes in flight/avoidance measures were much more common.

These findings suggest that flight/avoidance, although it obviously may occur as one component of a full pattern of defensive and emotional behaviors, is also somewhat separable from the others. When—as appears to be the case with TMT—it is the major or perhaps only consistent defensive behavior elicited, this may reflect a stimulus that is aversive or noxious but with little ability to predict the presence of threat or danger. That such stimuli fail to support rapid aversive conditioning suggests the need for a reanalysis of the characteristics required for an effective aversive US.

Keywords: Anxiety; Aversive conditioning; Avoidance; Behavioral assays; Cat odor; Defense; Defensive behavior; Flight; Rat; TMT

1. Introduction

The natural defensive behaviors of laboratory rats and mice have been evaluated in both seminatural and highly structured situations and characterized in terms of the relationship between particular behaviors and the stimuli that elicit and support them. Because a great deal of information is available on the antecedent and response aspects of defense, it has become possible to reliably elicit specific rodent defenses in an experimental context, providing consistent and of systematic information on their response to drugs and their relationships to other indices of emotion (Blanchard, 1997; Blanchard et al., 1997, 2003a).

In fact, an underlying assumption of this work has always been that defensive behaviors do reflect some aspect of emotion. This view is based on consistent findings linking them to behavior patterns that are accepted as indices of emotion. Thus, for example, the threat stimuli that most clearly and sharply elicit defensive behaviors, such as shock

Abbreviations: GAD, generalized anxiety disorder; MDTB, Mouse Defense Test Battery; TMT, trimethylthiazoline.

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or exposure to a predator, have residual anxiety-like effects in tests such as the elevated plus maze or defensive withdrawal (Adamec and Shallow, 1993; Adamec et al., 1997, 1998, 1999a,b; Steenbergen et al., 1990). In addition, defensive behaviors are easily and rapidly conditioned to both contexts and cues associated with noxious stimuli (Blanchard and Blanchard, 1969; Fanselow, 1980); particular defensive behaviors may be intimately involved in the conditioning process itself (Pinel and Mana, 1989), and specific defensive behaviors respond consistently and selectively to drugs effective against particular anxiety disorders, such as generalized anxiety disorder (GAD) or panic (Blanchard et al., 1989a,b, 2001a,b, 2003a).

This view that defensive behaviors reflect an emotional response has recently been challenged by findings that some, potentially threat relevant, stimuli may elicit defensive reactions that are not associated with rapid conditioning nor with anxiety-like behavior in other contexts. These findings provide the occasion for a reexamination of some long-standing assumptions about the relationships among aversive stimuli, emotions, stress, and conditioning.

2. Predator odors

Over the past 10 years, predator odors have come to be recognized as providing an important approach to the elicitation of defensive behaviors, fear, and anxiety in laboratory animals. This link has been validated by studies indicating that predator odor exposure, like exposure to the predator itself, can increase anxiety-like behavior on standard anxiety tests such as the plus maze, hole board, or social interaction tests (File and Zangrossi, 1993; Zangrossi and File, 1992a,b). Moreover, and again in parallel with findings of predator exposure (Blanchard et al., 1989a,b, 1998, 2001a,b; Griebel et al., 1995a,b,c, 1996, 1997, 1998), anxiolytic or potentially anxiolytic drugs have been shown to produce systematic changes in the behaviors elicited by cat skin/fur odors (Blanchard et al., 1990, 1993, 1997; Dielenberg and McGregor, 1999; McGregor and Dielenberg, 1999).

Because of the comparative ease of use of predator odors, rather than the living predator itself, these have come to be used frequently in research. Many early studies of predator odors, including all of those cited above, took these directly from the predator, collecting them by rubbing the animal or from a collar worn over a long period of time around the predator's neck. Predator hair has also come to be used as an odor source (Panksepp, 1998; Power and McGaugh, 2002). Recently, however, attention has shifted to the use of synthetic odors derived from feces or anal glands of predators such as weasels or foxes; research based on these odorants has proliferated during the very recent past (Anisman et al., 2001; Funk and Amir, 2000; Kavaliers et al., 1997; Morrow et al., 2000a,b; Perrot-Sinal and Petersen, 1997; Perrot-Sinal et al., 1999, 2000; Wallace and Rosen, 2000, 2001).

These studies have begun to suggest a considerable discrepancy between responses to some synthetic feces/anal gland odors and the cat fur/skin odors that were used in most of the earlier work involving odor stimuli. The two major predator fecal/anal gland odors that have been used in studies of defense are trimethylthiazoline (TMT) (synthetic fox fecal odor) and 2-propylthietane (synthetic weasel anal gland secretion). TMT, in particular, has been widely used in studies of predator odor effects on various aspects of emotionality in rats (Burwash et al., 1998a,b; Hotsenpiller and Williams, 1997; Morrow et al., 2000a,b; Perrot-Sinal et al., 2000; Vernet et al., 1992; Wallace and Rosen, 2000, 2001). However, it is notable that the behavioral effects of these feces/anal gland odorants may be considerably less clear and robust than are those of cat odor obtained by hair/skin contact with a live cat.

TMT is clearly repellent to rats (Vernet et al., 1992) and it, along with seven additional fecal/anal gland predator odors, reduced entry into, as well as consumption of food in, an odor-infused chamber (Burwash et al., 1998a). However, when tested in a field setting, TMT failed to alter any measures of location or movements of wild roof rats (*Rattus rattus*) (Burwash et al., 1998b). Morrow et al. (2000a) found that TMT exposure sufficient to enhance serum corticosterone failed to alter any of the behaviors (immobility, grooming, rearing, and lines crossed) measured in their study, although in the same report, all of these behaviors were significantly altered by a tone conditioned to foot shock. Wallace and Rosen (2000) reported TMT-elicited freezing that did not habituate over five 10-min sessions, although this was only substantial when animals were exposed in a very small enclosure. Hotsenpiller and Williams (1997) also reported freezing as well as analgesia to TMT. However, TMT failed to promote either cue + contextual conditioning (McGregor et al., 2002) or contextual conditioning even after five daily 10-min exposure sessions (Wallace and Rosen, 2000). Thus, in rats, TMT appears to be repellent or repugnant; it produces an analgesic reaction, corticosterone release, and some freezing/locomotion reductions; however, these changes do not appear to habituate or to foster aversive conditioning.

These findings may be contrasted with those obtained when rats are exposed to a predator or to the natural fur/skin odors of cats (the rat predator that has been used in the vast majority of studies involving actual predator–prey confrontation). When confronted by a domestic cat, rats show flight, avoidance, freezing, and risk assessment (Blanchard and Blanchard, 1971, 1972, 1989; Blanchard et al., 1989b) and reduced locomotion and nondefensive behaviors such as eating, drinking, exploration, and sexual activity (Blanchard and Blanchard, 1989: Blanchard et al., 1989b). These changes persist over a long period in the situation in which the encounter took place, suggesting that context conditioning has occurred (Blanchard and Blanchard, 1989). Like cat exposure, a single 10-min exposure to cat fur/skin odor elicits flight/ avoidance, freezing, and risk assessment (Blan-

Table 1
Defensive behaviors of rats during exposure to a cat or to odors associated with a predator stimulus

Rat defensive behavior	Live cat	Cat fur/skin odor	TMT
Avoidance	Yes (Blanchard and Blanchard, 1971, 1989; Blanchard et al., 1976, 1989a,b)	Yes (Blanchard et al., 1990, 1991, 1993, 2001b; Dielenberg and McGregor, 1999; McGregor et al., 2002)	Yes (Burwash et al., 1998a; Vernet et al., 1992) No (Burwash et al., 1998b, field study)
Eating reduction	Yes (Blanchard and Blanchard, 1989; Blanchard et al., 1989a,b)	Yes (Blanchard et al., 1990, 1991, 1993)	Yes (Burwash et al., 1998a)
Freezing/immobility	Yes (Blanchard and Blanchard, 1971, 1989; Blanchard et al., 1976, 1989a,b)	Yes (Blanchard et al., 1990, 1991, 1993, 2001; Dielenberg and McGregor, 1999; McGregor et al., 2002)	Yes (Hotsenpiller and Williams, 1997; Wallace and Rosen, 2000) No (Morrow et al., 2000a)
Locomotion reduction	Yes (Blanchard and Blanchard, 1971, 1989; Blanchard et al., 1976, 1989a,b)	Yes (Blanchard et al., 1990, 1991, 1993, 2001b; Dielenberg and McGregor, 1999; McGregor et al., 2002)	No (Morrow et al., 2000a)
Nondefensive behavior reduced	Yes (Blanchard and Blanchard, 1989; Blanchard et al., 1989a,b)	Yes (Blanchard et al., 1990, 1991, 1993, 2001b)	No (Morrow et al., 2000a)
Risk assessment	Yes (Blanchard and Blanchard, 1989; Blanchard et al., 1989a,b)	Yes (Blanchard et al., 1990, 1991, 1993, 2001b; Dielenberg and McGregor, 1999; McGregor et al., 2002)	No (McGregor et al., 2002)
Anxiogenic in elevated plus maze	Yes (Adamec and Shallow, 1993; Adamec et al., 1997, 1998, 1999a,b)	Yes (Dielenberg and McGregor, 1999; McGregor et al., 2002; Zangrossi and File, 1992a,b)	No (McGregor et al., 2002)
Supports conditioning	Yes (Blanchard and Blanchard, 1989; Blanchard et al., 1989a,b) (indirectly assessed by long-term behavior change in cat-exposure context)	Yes (Blanchard et al., 2001a,b; Dielenberg and McGregor, 1999; McGregor et al., 2002)	No (Blanchard et al., 2001b; McGregor et al., 2002; Morrow et al., 2000a; Wallace and Rosen, 2000)

chard et al., 1990, 1993, 2001b) in rats. It reduces nondefensive behaviors (Blanchard et al., 1997, 2001b; Dielenberg et al., 1999; Dielenberg and McGregor, 1999) and supports both cue and context conditioning (Blanchard et al., 2001b; Dielenberg et al., 1999; McGregor et al., 2002).

Exposure to a live cat enhances anxiety-like behavior in the elevated plus maze, a well-validated and extensively utilized test of anxiety-like responding (Adamec and Shallow, 1993; Adamec et al., 1997, 1998, 1999a,b). Similarly, cat fur/skin odor is anxiogenic in the elevated plus maze, light–dark, and social interaction tests (Dielenberg and McGregor, 1999; Zangrossi and File, 1992a,b). Comparison of the effects (Table 1) on rats of live cat versus cat fur/skin odor exposure indicates consistent agreement in a wide variety of defense-related measures, while similar studies using TMT are less consistent, with both positive and negative findings for some particular behavioral effects and consistent findings of no TMT effect on the majority of these measures. However, TMT does consistently elicit avoidance.

3. Conditioning of defensive responses to cat odor: TMT versus cat fur/skin odor

While these studies suggest that synthetic predator feces/anal gland odors may elicit a different and more restricted set of behavior changes than does cat fur/skin odor, there is more direct evidence of such a difference. McGregor et al. (2002) compared cat fur/skin odor and TMT within the same study. When rats in a test situation with a hide box in which they could shelter were confronted with either a cat fur/skin odor or TMT (each presented on pieces of a cloth cat collar), they hid in the shelter box; both groups showed sharp reductions in time near the odor stimulus compared to controls. However, only the cat odor exposed rats showed high levels of risk assessment (a defensive behavior to potential threat stimuli; Blanchard et al., 1991) to the odor stimulus, poking their heads out and visually inspecting the stimulus. When returned to the apparatus on the following day, the cat odor group showed less approach and activity but more head out behavior than the TMT or the control group. Thus, while both cat odor and TMT elicit aversion/avoidance of the odor stimulus, rats showed risk assessment to only the cat skin/fur odor stimulus. As risk assessment has been analyzed as an information-gathering process seen in the context of potentially dangerous stimuli (Pinel and Mana, 1989; Blanchard et al., 1991), this consistent association of cat fur/skin odor but not TMT with risk assessment suggests that only the fur/skin odor serves as a danger cue or danger signal stimulus. This study, like those of Wallace and Rosen (2000) and Morrow et al. (2000a,b) used high levels of TMT in the 30- to 100-μl range.

An additional set of studies involved a straight alley apparatus in which rats were exposed to relevant odorants. First, odor conditioning effects were demonstrated with a single 10-min exposure to cat fur/skin odor (Blanchard et al., 2001b). Second, four groups of rats were exposed to a cloth-covered odor block containing no added odor or 0.01, 0.05, or 0.1 μl TMT in the same apparatus and using the same habituation/exposure protocol (Blanchard et al., 2003b). TMT, at the doses typically used, is extremely pungent. It is clearly aversive to rats, i.e., eliciting turning away and avoidance (Vernet et al., 1992), and also repugnant and even nausea inducing to humans when in close contact (personal

observations). On the basis that potentially nauseating levels of TMT might have interfered with conditioning processes, this study (Blanchard et al., 2003b) used TMT levels as little as 1/10,000th of those used in previous studies. Nonetheless, these levels elicited significant increases in defensive behaviors, with contact durations with the odor block significantly lower for each of the TMT groups compared to controls, while the 0.05-μl TMT group also showed significantly less curve-back approach to the odor stimulus. However, during a conditioning test trial on the following day, when exposed to a no-odor block in the same apparatus, effects of TMT were not statistically significant for any measure.

4. Analysis of defensive behaviors and conditioning to fresh cat urine and feces

A third straight alley study (Blanchard et al., 2003b) using the same protocol attempted to determine why TMT and cat fur/skin odor have different effects on conditioning. A consistent failure to find TMT conditioning effects across a very wide range of doses and following multiple (Wallace and Rosen, 2000) as well as single exposures (McGregor et al., 2002; Blanchard et al., 2001b) had provided strong evidence that TMT does not support the rapid (i.e., single exposure) conditioning that is seen with cat fur/skin odor (McGregor et al., 2002). There are several points of difference between these stimuli that might be relevant, including source (cat vs. fox), synthetic (TMT) versus natural (cat fur/skin) stimuli, and type (cat fur/skin odor vs. feces odor). This study utilized only natural cat products, produced by the same cat on the same test day. Thus, differences in effects for the stimuli of different types could not be attributed to variation in the source species or to a synthetic/natural difference. Rats were exposed to a cloth-covered wooden block containing no added odor: 1 ml fresh cat urine; 1 g fresh cat feces; or cat fur/skin odor obtained by rubbing a cat with the cloth for 5 min (Blanchard et al., 2003b). During the exposure day, rats avoided both the feces and the fur/skin odor blocks compared to controls. However, on the following test day, only the fur/skin group showed significant avoidance (reduced curved-back approach and contact durations) of the (odorless) block, or significant freezing.

This finding in which cat feces can elicit both avoidance and freezing during exposure, but with no evidence of conditioning when rat subjects were replaced in the situation 24 h later, indicates that the source of a predator-related odor is important to its ability to support conditioning. This finding alone does not counterindicate a role for predator species in the effectiveness of predator odors, nor does it suggest that the synthetic versus natural difference between TMT and feces/anal gland odors is irrelevant to their ability to elicit a full pattern of defensive behaviors. It does suggest that, at least for rats confronted by cat odors, fur/skin odors are more effective than those of cat feces.

From an evolutionary perspective, it appears likely that this is due to the relative persistence of these odors. Small felids are stealth predators and their ability to creep up on prey depends, in part, on the lack of a strong odor by which they may be identified. In contrast, canids are typically cursorial predators and thus might not be expected to rely so heavily on odor reduction, nor should their (less controlled) odors be so important a danger-signal for potential prey. It is interesting that Panksepp (1998, p. 18) reports that the presence of cat fur reduced the play fighting of juvenile rats, but that fur from a Norwegian elkhound did not. Domestic cats spend about 8% of their waking time budget in oral grooming (Eckstein and Hart, 2000), an activity that may be important in reducing fur/skin odors. Oral grooming also leads to the ingestion of hair (as evidenced by hairballs in cats), further reducing environmental deposition of hair clumps that might give rise to more lasting fur/skin odors. In contrast, the most common source of feces odor is the presence of a fecal deposit. These materials are highly durable, and they may give off odors over a long period of time, even if partly buried (Pickett, 2000). Importantly, the animal that is the source of the fecal bolus does not need to be present for the odor to linger over a long period of time.

All of these considerations suggest that cat fur/skin odor and cat feces/anal gland odors are very different in the degree to which they predict the actual presence of a predator. The presence of cat fur/skin odor provides a strong indication that a cat is nearby and that it is nearby now. The presence of cat feces/anal gland odor indicates that a cat has been present. Thus, although both are cat-related odors, fur/skin odor is a straightforward danger cue, whereas feces/anal gland odors are not. Conditioning to the former should result in defensive responses to contextual and/or cue stimuli associated with this high-probability predator event, whereas conditioning to the latter would produce defensiveness to a context or cues that are much less likely to signal any actual danger. It is adaptive to be defensive to potential danger unless there is considerable evidence that the danger is not present. However, inappropriate defensiveness is also maladaptive due to the counteracting problem of optimal utilization of a time budget and a territory. Continued avoidance, freezing, or high-level risk assessment in a low-danger context may jeopardize other crucial activities such as foraging, nesting, or parenting. From this perspective, as the predictive power of a danger-cue stimulus declines, so should its ability to support conditioning.

This analysis does not indicate that aversive stimuli that are poorly predictive or nonpredictive of danger should be incapable of supporting conditioning under any circumstances. Present data for TMT, for example, have involved a maximum of five daily 10-min exposures (Wallace and Rosen, 2000). While this produced no signs of context conditioning, it does not indicate that 50 such exposures would have no effect. However, such studies (McGregor et al., 2002; Blanchard et al., 2003b) do indicate that TMT does not support rapid context conditioning, nor do cat feces

odors (Blanchard et al., 2003b) or aversive but nonpredictive odors such as triethylamine or formaldehyde (McGregor et al., 2002); whereas a more predictive stimulus, cat fur/skin odor produces both cue and context conditioning in a single such exposure (Blanchard et al., 2001b; McGregor et al., 2002). Certainly, further work involving other highly or poorly predictive predator stimuli and additional aversive odors that do not predict danger is necessary to determine if the variable of danger predictiveness is crucial in this relationship. Indeed, it is possible that the relationship may be somewhat different for species other than the rat, perhaps due to their different mix of defensive behaviors. However, the present data support the view that in rats, rapid conditioning is associated with cues that are highly predictive of danger and not with less predictive cues.

5. Anxiety and defense differences of fur/skin versus feces/anal gland odors

An additional feature of cat fur/skin odor that appears to be different for predator feces/anal gland odors is the ability to elicit an emotional response in a different context. As noted earlier, exposure to an actual predator does produce enhanced anxiety-like behavior in the elevated plus maze (Adamec and Shallow, 1993, Adamec et al., 1997, 1998, 1999a,b). Exposure to cat fur/skin odor has the same effect, again enhancing EPM anxiety-like behavior (Dielenberg and McGregor, 1999; McGregor et al., 2002; Zangrossi and File, 1992a,b). TMT does not (McGregor et al., 2002).

Cat exposure also consistently elicits risk assessment activities in rats (Blanchard and Blanchard, 1989; Blanchard et al., 1989a,b), as does cat fur/skin odor (Blanchard et al., 1990, 1993, 2001a,b; McGregor et al., 2002). In contrast, McGregor et al. (2002) have reported that rats do not show risk assessment to TMT. Both phenomena are potentially relevant to conditioning, as risk assessment has been analyzed as an information-gathering activity leading to learning about aversive stimuli (Blanchard et al., 1991), whereas anxiety-like behavior may be related to motivational aspects of responsivity to danger-cue stimuli.

If odorants elicit anxiety-like reactions, then antianxiety drugs should reduce these. The effects of a number of relevant drugs on rat responses to cat fur/skin odor have been summarized by Dielenberg and McGregor (2001). Based on studies from three different laboratories, midazolam, diazepam, chlordiazepoxide, ethanol, chronic imipramine, and chronic fluoxetine all reduce defensiveness to cat fur/skin odor. In contrast, midazolam has no effect on avoidance of TMT (Dielenberg and McGregor, 2001; McGregor et al., 2002).

We have recently tested (Table 2; unpublished data) a number of additional antianxiety compounds in a staircase test, with both cat fur/skin odor and TMT, compared to a brush with no odor added. The brush was placed on the top stair. Although there was no difference in contact time with the brush for the cat fur/skin odor and TMT groups, only the cat fur/skin odor group responded to acute doses of clinically effective anxiolytics by increasing contact with the brush. The anxiogenic effect of fluoxetine reflects that an acute dose was used, whereas chronic administration (3 weeks) reduced defensiveness to cat fur/skin odor (Dielenberg and McGregor, 2001). This is consistent with previous findings for the effects of acute versus chronic fluoxetine on defensive behavior (Griebel et al., 1995a).

Table 2
Anxiety-modulating action of several anxiolytics in the staircase test containing (1) a brush (neutral odor), (2) a cat odor-saturated brush, or (3) a TMT-saturated brush

Compound	Action/class	Neutral odor	Cat odor	TMT
Diazepam	Benzodiazepine	+	+	o
Fluoxetine	SSRI	−	−	−
Imipramine	Tricyclic	o	+	o
Buspirone	5-HT_{1A} agonist	o	+	o
Antalarmin	CRF_1 antagonist	o	+	o

+, Anxiolysis; −, anxiogenesis; o, no effect.

These data provide a consistent indication that the defensive effects of cat fur/skin odor are mediated by anxiety, whereas the defensive (avoidance) effects of TMT are not. This interpretation is consistent with findings that cat fur/skin odor, but not TMT, produces residual anxiety-like behavior in the elevated plus maze, and it further suggests that the failure of TMT to support conditioning of defensiveness to contexts with which it was associated may reflect the failure of this compound to elicit an emotional response.

6. Defensive behaviors in the mouse/defense test battery

Relevant to analysis of the relationship among defensive behaviors, emotionality, and potentially learning, a body of data has been collected (Blanchard et al., 2003a) in the Mouse Defense Test Battery (MDTB) on the responses of mice to encounters with a predator and afterwards to the context in which the predator appeared. The MDTB is frequently used in the context of drug evaluation, and the systematic body of information that is emerging is much more extensive than for tests using rat subjects.

In the MDTB, mice first are chased by a (hand-manipulated) anesthetized predator, an adult male rat, in a runway permitting endless forward locomotion. Flight/avoidance and risk assessment (stopping and orienting toward the chasing rat) are the major defenses seen in this chase/flight situation. Following the closure of doors to exclude flight, the predator is held briefly at a fixed distance from the mouse. During this period, the latter tends to show some immobility, alternating with approaching and then withdrawing from the threat stimulus. These approach/withdrawal behaviors represent another example of risk assessment or checking out the threat, albeit in a different situation and in the context of a different specific defensive behavior than

occurs while the mouse is fleeing in the chase/flight test. As the approaching predator comes closer to the mouse, the latter may show an upright stance and sonic vocalization (defensive threat) and then defensive attack (jump attack and biting) as contact becomes imminent.

A final measure involves the difference between the mouse subject's behavior in a 3-min period in the apparatus prior to introduction of the predator and the behavior seen after the predator has been removed. Typically, whereas no systematic escape attempts were made prior to predator exposure, the mouse makes repeated escape jumps at the walls of the apparatus after predator testing is concluded. These behaviors, labeled "contextual defense," may either represent a conditioned response to the contextual cues of the apparatus or some type of residual emotional response to predator exposure, potentially providing a parallel to the enhanced anxiety-like behavior of rats in the elevated plus maze following predator (or cat fur/skin odor) exposure (Adamec and Shallow, 1993; Adamec et al., 1997, 1998, 1999a,b; McGregor et al., 2002; Zangrossi and File, 1992a,b). As this anxiety-like behavior is measured in a situation different from the one in which subjects encountered the predator stimulus, it is not a conditioned response to a context paired with threat but a residual emotional reaction. Since the contextual stimuli of the MDTB are the same as those associated with predator exposure, contextual defense may or may not reflect conditioning.

Griebel et al. (1996) have further analyzed risk assessment measures of the MDTB as reflecting cognitive aspects of anxiety, whereas defensive threat/attack measures reflect a more emotional component. If contextual defense does reflect some type of emotional response to predator exposure, then a relationship to defensive threat/attack would be expected. If it reflects conditioning, then risk assessment may be another important mediator of this response. A list of 70+ drugs evaluated in the MDTB, along with their effects on flight measures, risk assessment measures, defensive threat/attack measures, and on contextual anxiety, is given in Blanchard et al. (2003a). This list confirms previous analyses indicating that risk assessment and defensive threat/attack—both initially identified as responding to drugs effective against GAD in earlier rat studies (Blanchard et al., 1989a,b)—continue to show a strong response to such drugs in the MDTB, and that contextual anxiety is also responsive to these GAD-effective drugs (Blanchard et al., 2001a,b).

One way of examining this potential relationship is to analyze drug effects that are discordant for risk assessment/defensive threat/attack and contextual defense. Disregarding some four drugs that produced enhancements rather than reductions in various measures, 21 drugs were discordant for risk assessment/defensive threat/attack effects and effects on contextual defense. These consist of drugs that either reduced risk assessment and defensive threat/attack and left contextual defense intact or reduced contextual defense while leaving the others intact. Interpretations of these two types of discordance may be very different, given that baseline (control) levels reflect an animal responding to a predator and showing effects on all of these measures. If contextual defense reflects a cognitive/attentional and emotional response to the predator, it should be very difficult for an independent variable manipulation to reduce it without altering the other responses. This suggests that findings that drugs seldom reduce contextual defense without altering either risk assessment or defensive threat/attack should be interpreted as indicating the dependence of contextual defense on the cognitive and emotional mechanisms underlying these behaviors. However, given the possibility of a threshold such that risk assessment and defensive threat/attack might require to be substantially reduced before similar effects would occur in contextual defense, examples of discordance in the opposite direction would not necessarily weaken the case that contextual defense depends on cognitive and/or emotional response to predator threat. This "effect subtracting" model is opposite to an "effect adding" model such as might be appropriate if the independent variable was enhancing measures from a zero baseline rather than reducing them from a clearly superthreshold baseline.

This is precisely what was found. Of the 21 discordant drug effects, 20 reduced either risk assessment measures or defensive threat/attack measures or both but produced no change in contextual defense at the highest dose given. However, the opposite relationship of a reduction in contextual defense measures without any reductions in risk assessment/defensive threat/attack measures was extremely rare, occurring with only one drug (phenelzine, given on an acute basis). This pattern suggests that changes in risk assessment/defensive threat/attack are important, perhaps crucial, mediators of changes in contextual defense. However, for reductions in risk assessment or defensive threat/attack to impact contextual defense, some type of threshold of reduction in effect may be necessary. These data also suggest the possibility that reductions in contextual defense with different drugs may potentially reflect different neural mechanisms, depending on whether cognitive/attentional or emotional mechanisms are the major mediating elements.

However, the relationship between flight and contextual defense was somewhat different. A total of 23 drugs showed discordant effects for flight and contextual defense, reducing one set of measures but producing no change in the other. Seventeen drugs producing a change in flight failed to impact contextual defense, while six drugs had no effect on flight but mildly to strongly reduced contextual defense at dose levels that were in some cases much smaller than those that did not impact flight. It is notable that all six of these drugs also reduced either risk assessment or defensive threat/attack. These effects were consistent within drug classes in that three $5HT_{1A}$ agonists (8-OH-DPAT, buspirone, and gepirone) produced no flight changes while sharply reducing contextual defense (and defensive threat/attack, as well).

Thus, in contrast to a single example of contextual defense reductions unaccompanied by changes in risk

assessment or defensive threat/attack, there were six drugs that reduced contextual defense without reducing flight, suggesting flight is less directly related to this contextual defense response. While this analysis is clearly consistent with earlier interpretations (Blanchard et al., 2001a) that flight is particularly responsive to drugs effective against panic disorder rather than to the anti-GAD drugs that impact risk assessment, defensive threat and attack, and contextual defense, it particularly draws attention to an apparent lack of relationship between flight measures and contextual defense.

These MDTB findings present a number of potential parallels to the TMT/feces results. TMT and feces produce avoidance. They do not support conditioning. TMT (feces have not been evaluated in these specific contexts) does not elicit risk assessment or support a residual emotional reactivity. An apparent sticking point is that the rubric for behavior in the MDTB that corresponds to these relationships is flight, not avoidance, but this is easily resolved: MDTB ''flight'' includes measures of avoidance (e.g., distance between the subject and predator when avoidance occurs) as well as measures of flight distance and flight speed. Thus, both flight and avoidance measures are included in the ''flight'' composite.

An additional question is how, if the MDTB ''flight'' category responds selectively to drugs active against panic disorder, these findings might be relevant to the common clinical association between panic attacks and subsequent agoraphobia. This association suggests that panic attacks may produce a conditioned avoidance of the situation(s) in which they have occurred. In contrast, the TMT/feces and MDTB data suggest that flight/avoidance per se may not result in a conditioned response to such situations. While a detailed discussion of the association between panic and agoraphobia in terms of a flight-conditioning model is far outside the scope of this article, some recent observations may be of interest. First, the lifetime rate for panic attack is much higher than that for agoraphobia, suggesting that conditioning of avoidance to the panic context does not occur with single experiences (Wittchen and Essau, 1993). Second the route from panic attack to agoraphobia typically does not reflect a specific emotional response to a place in which a panic attack previously occurred, but instead a restriction of activity to ''safe'' areas and an avoidance of many ''public places''—not just those in which panic attacks occurred (Langs et al., 2000). Third, agoraphobia appears to be particularly associated with fear of social consequences such as being thought crazy or of embarrassment (Langs et al., 2000) rather than a conditioned emotional response to a nonsocial context. These considerations suggest that the association between panic attacks and agoraphobia does not reflect simple emotional conditioning to the specific context in which the attacks occurred, and raise a further issue of what specific circumstances are necessary for such context conditioning as this may not be a common sequela of panic attack.

7. Summary

The use of predator-related stimuli provides data suggesting a potential division between two systems of response to aversive events. Evaluation of aversive stimuli that serve as high probability danger cues and those that do not suggests that these may produce very different patterns of both unconditioned and conditioned behaviors. Danger-cue stimuli elicit a trio of emotional responses including rapid context conditioning, residual emotional reactivity, and persistent risk assessment activity. This pattern seen in response to predator exposure (Adamec and Shallow, 1993; Adamec et al., 1997, 1998, 1999a,b; Blanchard and Blanchard, 1989) also occurs to cues (cat fur/skin odor) that strongly suggest the presence of a predator (Blanchard et al., 2001a,b; McGregor et al., 2002). In contrast, predator-associated stimuli that do not strongly suggest predator presence, and also aversive odors having no association with predators, may elicit strong avoidance but not the trio of emotion-related responses (Blanchard et al., 2003b; McGregor et al., 2002). This effect does not appear to reflect the magnitude or intensity of the eliciting stimulus since the failure to find conditioning with one such stimulus, TMT, has been consistently reported at widely varying dose levels that all elicit avoidance.

The MDTB enables a very different analytic approach. The substantial number of drugs that have been used in this test may now begin to provide a pharmacological ''scalpel'' by which drug effects on specific defensive behaviors can be isolated and their relationships to other behaviors evaluated. One such analysis suggests that contextual defense, which may reflect either context-conditioned defensiveness or a residual emotional response, is almost never reduced in response to drugs unless those drugs have attenuated risk assessment or defensive threat/attack responses. However, context conditioning is more often reduced in response to drugs that do not alter flight/avoidance responses. The ability of drug effects to dissociate contextual defense from flight/avoidance is consonant with a view that this category of response, potentially related to the avoidance responses consistently elicited by aversive but non-danger-cue stimuli, may reflect a different and more restricted aversive response than that seen to dangerous or danger-cue stimuli. This is not to say that flight and avoidance are not components of the total pattern of defensiveness that is elicited by high-level threat such as a predator. They clearly are. However, flight/avoidance appears to be more drug dissociable from other components of this pattern than are risk assessment or defensive threat/attack.

These data suggest the value of reanalysis of the essential characteristics of unconditioned stimuli that support rapid aversive conditioning. A core problem is embedded in the name ''a verso,'' which literally means turning away from, i.e., avoidance. However, when avoidance is the only element of a defensive response to a stimulus, that stimulus appears to be less likely to support rapid conditioning than is one that elicits a broader range of defensive and emotional behaviors.

The finding that this pattern may occur with predator feces, as well as synthetic predator stimuli, suggests that the evolved ability of the aversive stimulus to predict danger may be a major determinant of whether it will elicit the full range of emotional and conditioning behaviors in rats.

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