The Benzodiazepine Alprazolam Dissociates Contextual Fear from Cued Fear in Humans as Assessed by Fear-potentiated Startle

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Background: *The startle reflex is potentiated by aversive states. It has been proposed that phasic startle potentiation to a threat cue and sustained startle potentiation to contextual stimuli reflect distinct processes mediated by different brain structures. The present study tested the hypothesis that alprazolam would reduce the sustained startle potentiation to contextual threats but not the startle potentiation to a threat cue.*

Methods: *Sixteen healthy subjects received each of four treatments: placebo, .5 mg of alprazolam, 1 mg of alprazolam, and 50 mg of diphenhydramine (Benadryl) in a crossover design. Participants were exposed to three conditions, including one in which predictable aversive shocks were signaled by a cue, a second in which shocks were administered unpredictably, and a third condition in which no shocks were anticipated. Acoustic startle were delivered regularly across conditions.*

Results: *Phasic startle potentiation to the threat cue in the predictable condition was not affected by alprazolam. In contrast, the sustained increase in startle in the predictable and unpredictable conditions was reduced significantly by the high dose of alprazolam.*

Conclusions: *Startle responses to an explicit threat cue and to an aversive context are psychopharmacologically distinct, suggesting that they may represent functionally dissociable aversive states.*

Key Words: Alprazolam, anxiety, benzodiazepine, context, fear, startle reflex

The startle reflex, a cross-species response to a sudden intense stimulus, is sensitive to aversive states (Davis et al 1993). Rodents show robust startle potentiation to a conditioned cue that signals an aversive event (e.g., a shock). Evidence suggests that this effect is mediated by the central nucleus of the amygdala (CeA). For example, lesions of the CeA block fear-potentiated startle to a conditioned cue previously paired with shock (Davis 1998). Although the CeA responds to various types of stressors, this structure is not always critical for fear-potentiated startle (Davis 1998) or for responses to stress in general (Hammack et al 2004). Davis and his collaborators have reported a series of studies showing no effect of lesions of the CeA on startle potentiation caused by various stressors, such as bright lights (i.e., light-enhanced startle), shock sensitization, and corticotropin-releasing hormone injection (reviewed in Walker et al 2003). Rather, another structure, the bed nucleus of the stria terminalis (BNST), was found to mediate startle potentiation in these conditions (Walker et al 2003). In an analysis of the experimental situations that do or do not require the CeA and BNST, Walker et al (2003) suggested that these structures were involved in functionally different aversive states in rodents. It was proposed that the CeA was crucial for the phasic form of fear-potentiated startle to a predictable threat cue, whereas the BNST was responsible for the more sustained form of startle potentiation induced by unpredictable or unconditioned aversive stimuli (Davis 1998; Gewirtz et al 1998; Walker and Davis 1997a). It was suggested further that the two aversive states mediated by the CeA and the BNST in rodents were reminiscent of fear and anxiety states in humans, respectively (Davis 1998). According to this view, fear is a response to a clearly identifiable danger that subsides shortly after the offset of a threat cue. Anxiety is a more sustained form of general distress and anxious apprehension in response to less identifiable cues (Barlow 2000; Davis 1998; Lang et al 2000).

The distinction between a phasic and a more sustained form of startle potentiation also has been made in humans (Cuthbert et al 2003; Grillon et al 1991, 1997; Grillon and Davis 1997; Lang et al 2000; Pole et al 2003). Startle is potentiated by an explicit threat cue that signals an impending aversive event (e.g., a shock; Grillon et al 1993; Hamm and Vaitl 1996), such as when phobic individuals are confronted with their phobic objects (de Jong et al 1996; Globisch et al 1999). More sustained forms of startle potentiation can be found among individuals who are exposed to stressful experimental settings (Bocker et al 2001; Grillon and Ameli 1998; Pole et al 2003), when the experimental room is in complete darkness (Grillon et al 1997), or after context conditioning (Grillon and Davis 1997).

Consistent with the animal literature, phasic and sustained forms of startle potentiation in humans appear to reflect distinct processes. For example, individuals with posttraumatic stress disorder (PTSD) or with panic disorder display normal startle to explicit threat cues that signal a shock but display enhanced startle reactivity in the experimental context in which the shocks are administered (Grillon et al 1994, 1998b; Pole et al 2003). Patients with PTSD also exhibit increased context conditioning (Grillon and Morgan 1999) and increased facilitation of startle in the dark (Grillon et al 1998a).

There currently is little information on the neurobiological mechanisms that may differentiate phasic cued fear from more sustained contextual anxiety in humans. Evidence for such a neurobiological dissociation would be bolstered if one could demonstrate that these two forms of aversive states are differentially responsive to psychopharmacologic treatments. The main objective of this study was to obtain such evidence by using the benzodiazepine alprazolam.

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There is an emerging literature on the effect of benzodiazepines on fear-potentiated startle in humans. We have reported results of four studies showing that oxazepam and diazepam did not affect fear-potentiated startle to a threat cue (Baas et al 2002). In contrast, other groups reported that alprazolam (Riba et al 2001), diazepam (Bitsios et al 1999), and lorazepam (Graham et al 2005) reduced fear-potentiated startle in threat of shock experiments. However, it is not clear whether the reduction in fear-potentiated startle in these latter studies was caused by an anxiolytic effect per se or was an artifact of the sedative effect of benzodiazepines on baseline startle. A drug-induced reduction in baseline startle reactivity may lead to an inaccurate measurement of fear-potentiated startle (Grillon and Baas 2002; Walker and Davis 2002b). Important methodologic differences between studies also may explain contradictory findings. For example, in two studies the subjects were verbally informed of the threat and safe conditions while the shock electrodes were being attached or removed, and these studies were conducted in near darkness (Bitsios et al 1999; Graham et al 2005). It has been argued that both the shock electrodes and darkness are contextual stimuli (Grillon and Ameli 1998; Grillon et al 1997; Walker and Davis 1997b), which may have increased contextual anxiety. Hence, it is unclear whether the response that was effectively reduced by diazepam in the Bitsios et al (1999) design constitutes a cued fear response or contextual anxiety caused by placement of the shock electrodes.

There is evidence to suggest that benzodiazepines affect contextual anxiety. In rodents, benzodiazepines reduce baseline startle. This reduction is a result not only of sedation but also of an anxiolytic effect on contextual fear (Guscott et al 2000). Baas et al (2002) reported a similar effect in humans. In addition, Baas et al (2002) showed that diazepam reduced the facilitation of startle in the dark in humans. The facilitation of startle in the dark in humans is a sustained form of startle potentiation similar to the light-enhanced startle in rodents (Grillon and Baas 2002), which itself is alleviated by benzodiazepines (Walker and Davis 2002a; de Jong et al 2002).

The present study improved on past studies by addressing two main issues. First, we compared fear-potentiated startle elicited by predictable and unpredictable shocks to clearly dissociate phasic cued fear from sustained anxiety (Grillon et al 2004). Second, a sedative drug (diphenhydramine) that is not used for anxiolysis was included to control for confounding effects of sedation on startle reactivity (Grillon and Baas 2002; Walker and Davis 2002b). The experimental design was based on the observation that unpredictable aversive events increase context conditioning (Grillon and Davis 1997; Odling-Smee 1975), which can be conceived of as a form of sustained anxiety. In this design, subjects are presented with three conditions: no shocks, predictable or signaled shocks, and unpredictable or nonsignaled shocks. Previous results show two types of aversive responses: (1) a phasic startle potentiation during the threat signal relative to the absence of threat signal in the predictable shock condition and (2) a sustained startle potentiation during the predictable and the unpredictable shock condition compared with the no-shock condition in the absence of specific cues (Grillon et al 2004). For the remainder of this article, cued fear will refer to the phasic startle potentiation to the explicit threat signal, and contextual anxiety will refer to the sustained startle potentiation in the absence of cues.

The main hypothesis of the study was that alprazolam would not affect fear-potentiated startle to the threat signal in the predictable shock condition (cued fear) but would reduce fear-potentiated startle in the absence of cues in the predictable and unpredictable shock condition (contextual anxiety). Each subject was tested in four treatments: (1) placebo, (2) a low dose (.5 mg) of alprazolam, (3) a high dose (1 mg) of alprazolam, and (4) diphenhydramine (Benadryl, 50 mg). The two doses of alprazolam were selected on the basis of a study that reported a reduction of fear-potentiated startle by alprazolam (Riba et al 2001).

Methods and Materials

Participants

Participants were healthy volunteers who gave written informed consent that had been approved by the National Institutes of Mental Health Human Investigation Review Board. Inclusion criteria included the following: (1) no past or current psychiatric disorders as per Structured Clinical Interview for DSM-IV (SCID; First et al 1995), (2) no medical condition that interfered with the objectives of the study as established by a physician, and (3) no use of illicit drugs or psychoactive medications as per urine screen.

Participants underwent a screening session that consisted of a SCID, a physical exam, and a shock workup procedure to establish a level of shock that was "highly annoying but not painful". The mean intensity of the shock was 4.2 μA, with a range of 3–5 μA. In addition, subjects were screened for baseline startle reactivity with nine startle stimuli (40-ms duration, 103 dB). Subjects who displayed small startle responses (a mean of less than 5 μV over nine startle responses) or displayed no startle response on at least one trial were not invited to participate in the study. Four to 10 days after screening, participants returned for the first of four testing sessions. Sixteen subjects (five were male) with a mean age of 23.0 years (SD = 4.7 y) ultimately were included in the study. Mean scores on the state and trait portions of Spielberger's State and Trait Anxiety Inventory (Spielberger 1983) were 26.0 (SD = 4.0) and 28.2 (SD = 4.8), respectively.

Drug Manipulation

The treatments were placebo, .5 mg of alprazolam, 1 mg of alprazolam, and 50 mg of diphenhydramine, tested in a double-blind crossover design (within subjects). Treatment administration was performed according to a randomization table comprising a 4 × 4 Latin square repeated four times.

Procedure

On the test day, subjects filled out a mood rating scale (pretreatment) that evaluated subjective feelings of mental and physical sedation. Next, they ingested a capsule containing one of the active drugs or placebo. Subjects rested for 1 hour to allow drug absorption, after which the procedure to apply measurement- and shock-electrodes was started.

Details of the procedures are provided elsewhere (Grillon et al 2004). During testing, subjects first were presented a habituation block consisting of nine startle stimuli delivered every 18–25 sec to reduce excessive initial startle reactivity before the threat study (data not reported). Participants then were given explicit instructions regarding the conditions under which they would and would not receive an aversive event. After the instructions, the threat experiment began. The experiment consisted of three different conditions: no shock (N), predictable shock (P), and unpredictable shock (U), each lasting approximately 150 sec. In the N condition, no shocks were delivered. In the P condition, shocks were administered predictably, that is, only in the presence of a threat cue. In the U condition, the shocks were

Table 1. Mean (SEM) Startle Magnitude (μV) during the Cue and during ITI across Treatments and Conditions

	Neutral		Predictable		Unpredictable	
	Cue	ITI	Cue	ITI	Cue	ITI
Placebo	38.3 (9.7)	38.4 (10.8)	57.9 (11.9)	47.2 (11.1)	55.1 (12.1)	53.6 (12.0)
Diphenhydramine	28.0 (11.7)	26.8 (10.8)	42.2 (12.6)	32.2 (12.0)	40.1 (12.5)	40.3 (13.0)
Alprazolam, low	39.4 (10.0)	38.2 (10.6)	51.9 (12.3)	41.6 (10.0)	57.0 (12.3)	55.7 (12.8)
Alprazolam, high	23.9 (7.6)	25.5 (8.0)	37.0 (9.3)	26.3 (7.2)	33.0 (8.3)	30.3 (7.9)

ITI, intertrial intervals.

unpredictable. In each 150-sec condition, an 8-sec cue was presented four times. The cues were different geometric colored shapes in each condition (e.g., a blue square for N, a red circle for P, and a green star for U). The cues signaled the possibility of receiving an aversive stimulus only in the P condition. They had no signal value in the N and U conditions. Instructions were displayed on a computer monitor to inform participants of the current condition by displaying the following information throughout the testing procedure: "no shock" (N), "shock only during shape" (P), or "shock at any time" (U). During each predictable and unpredictable condition, one shock was administered. When a shock was administered, it was delivered during the cue in the predictable condition and in the absence of the cues in the unpredictable condition. In each N, P, and U condition, six acoustic startle stimuli were delivered, three during intertrial intervals (ITI; i.e., between cues) and one during three of the four cues, 5–7 sec after cue onset. The threat experiment consisted of two recording blocks with a 5- to 10-min rest between blocks. Each block consisted of three N, two P, and two U conditions in one of the following two orders: P N U N U N P or U N P N P N U. Each participant was presented with the two orders, with half the participants starting with the P condition. One shock was administered in each individual P and U condition, for a total of four shocks in the four P conditions and of four shocks in the four U conditions. The shock was delivered 7.5 sec after cue onset in the P condition. It was administered either 7 sec or 10 sec after cue offset in the unpredictable condition. No startle stimuli could follow a shock by less than 10 sec.

Subjects were asked to fill out the mood rating scale another time during the interval between the two threat blocks. In addition, after each recording block, subjects retrospectively rated how anxious they felt in the presence and absence of the cue in each condition (N, P, U) on an analog scale ranging from 0 (not at all anxious) to 10 (extremely anxious).

Stimuli and Physiological Responses

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments, London, United Kingdom). The acoustic startle stimulus was a 40-ms duration, 103-dB(A) burst of white noise with a near-instantaneous rise time, presented binaurally through headphones. The eyeblink reflex was recorded with electrodes placed under the left eye. Amplifier bandwidth was set to 30–500 Hz. The electric shock was produced by a constant current stimulator and was administered on the right wrist.

Data Analysis

Peak amplitude of the blink reflex was determined in the 20- to 100-msec time frame after stimulus onset relative to baseline (average baseline EMG level for the 50 ms immediately preceding stimulus onset) and was averaged within each condition. The startle data and retrospective measures of subjective anxiety were analyzed with analyses of variance (ANOVA) with repeated measures. Preliminary analyses indicated no gender difference for the startle and subjective measures. Hence, gender was not entered as a factor in the data analysis. Given our specific a priori hypotheses, separate comparisons were conducted to examine cued fear and contextual anxiety. Cued fear was tested by first calculating the difference scores between startle magnitude during the cues and startle magnitude during ITI. These difference scores then were analyzed with two-way ANOVAs, with treatment (placebo, diphenhydramine, low alprazolam, or high alprazolam) and condition (N, P, or U) as repeated factors. Contextual anxiety was evaluated by using the startle magnitudes during ITI in each of the three conditions. This involved two-way ANOVAs, with treatment (placebo, diphenhydramine, low alprazolam, or high alprazolam) and condition (N, P, or U) as repeated factors. Given a significant main effect of condition, the presence of a linear trend in startle magnitude over N, P, and U was tested. It was hypothesized that the linear trend would be affected by alprazolam but not by diphenhydramine, compared with placebo. These analyses were repeated on standardized scores by using within-subjects t scores ([Z scores × 10] + 50). Because similar results were obtained with the raw scores and with the t scores for within-subjects comparisons, only results of the raw scores are presented. The same analysis was conducted for the retrospective subjective reports of anxiety. Alpha was set at .05 for all statistical tests. Greenhouse-Geisser corrections (GG-ϵ) were used for main effects and for interactions involving factors with more than two levels.

Results

Startle Data

Cued Fear. The results in each condition and in each treatment are presented in Table 1. Figure 1A displays the magnitude of fear-potentiated startle (cue minus ITI). There was a significant main effect of condition [$F(2,30) = 11.9$, $p = .001$, GG-$\epsilon = .65$], reflecting greater fear-potentiated startle during the cue in the U condition, compared to the N and P condition. These effects were not affected by treatment, as reflected by a lack of treatment main effect [$F(3,45) = .03$, ns] and treatment × condition interaction [$F(6,90) = .3$, ns].

Contextual Anxiety. The ITI data that were used to evaluate contextual anxiety are shown in Table 1. Consistent with our previous report (Grillon et al 2004), there was a linear increase in startle magnitude from the neutral, to the predictable, to the unpredictable condition [main effect of condition: $F(2,30) = 14.0$, $p < .0009$, GG-$\epsilon = .7$; linear effect of condition: $F(1,15) = 18.7$, $p < .0009$]. There also was a trend for a main effect of treatment [$F(3,45) = 2.4$, $p = .09$, GG-$\epsilon = .8$]. However, this effect was qualified by a significant treatment × condition interaction [$F(6,90) = 3.4$, $p < .01$, GG-$\epsilon = .7$] and by a treatment × condition linear effect [$F(1,15) = 5.7$, $p < .03$]. These

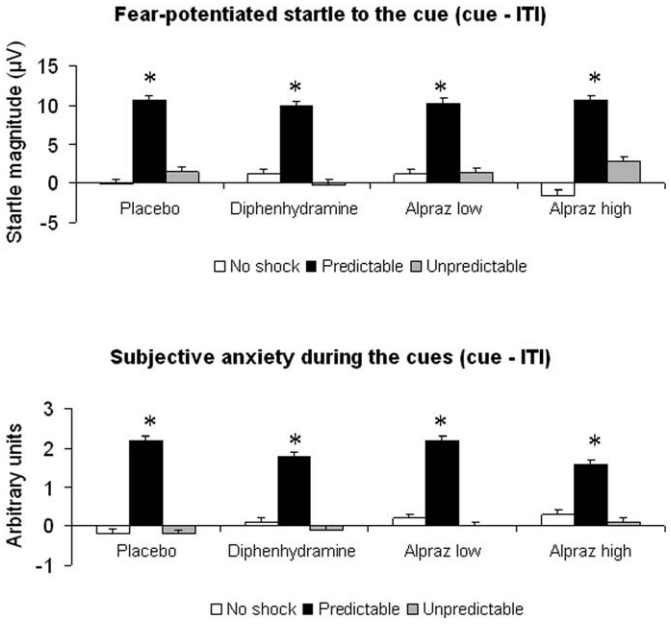

Figure 1. Responses to the cues in each treatment expressed as a change from baseline. **(A)** Cued fear-potentiated startle. Difference scores reflecting cue minus ITI startle magnitudes in the no shock, predictable, and unpredictable conditions. The main effect of condition was significant. *Significantly ($p < .05$) increased startle from ITI. There was no significant difference among treatment. **(B)** Subjective anxiety. Difference scores reflecting startle magnitudes in the presence minus absence of cues in each condition. The main effect of condition was significant. *Significantly ($p < .05$) increased startle from ITI. There was no significant difference among treatments. Alpraz,alprazolam.

interactions reflect differential effects of treatment on startle potentiation during ITI. Follow-up tests focused on the increase in startle magnitude from the neutral to both the predictable and unpredictable conditions across treatments. This analysis was implemented by calculating the difference scores for predictable minus no-shock and unpredictable minus no-shock conditions (Figure 2A). These difference scores were entered into a treatment (placebo or high alprazolam) × condition (P or U) ANOVA yielding a main effect of treatment [$F(1,15) = 10.2$, $p < .0009$] and no significant treatment × condition interaction. The significant treatment main effect confirmed that the ITI startle potentiation from the neutral to both the predictable and unpredictable condition was reduced by high alprazolam.

The active control substance diphenhydramine was used to examine whether the treatment effect could be attributed solely to sedation. This was not the case. First, the treatment (placebo or diphenhydramine) × condition (P or U) ANOVA showed no significant effect involving treatment. Second, the treatment (high alprazolam or diphenhydramine) × condition (P or U) ANOVA showed a significantly lower potentiated startle in the high-alprazolam condition compared to diphenhydramine [$F(1,15) = 5.5$, $p = .03$]. The placebo, diphenhydramine, and low-alprazolam treatment conditions did not differ significantly from each other. Note that high alprazolam also reduced potentiated startle compared with the low-alprazolam treatment condition [$F(1,15) = 5.7$, $p = .03$].

Retrospective Ratings of Anxiety

Cued Fear. The anxiety rating scores are shown in Figure 1B. There was a significant main effect of condition [$F(2,30) = 56.0$, $p = .0009$, GG-$\epsilon = .95$], reflecting greater subjective anxiety during the cue in the P condition, compared to the N and U conditions. There was no significant main effect of treatment [$F(3,45) = .61$], but the treatment × condition interaction was significant [$F(6,90) = 2.4$, $p < .04$]. Because the comparison of interest was anxiety ratings in the P condition across treatments, follow-up tests contrasted anxiety scores between treatments in the P condition in a one-way ANOVA. The results indicated no reduction in anxiety with any of the treatments as reflected by a nonsignificant treatment main effect [$F(3,45) = 1.9$, $p > .1$].

Contextual Anxiety. Consistent with the startle data, there was a progressive increase in anxiety from the neutral, to the predictable, to the unpredictable condition [$F(2,30) = 56.7$, $p < .0009$, GG-$\epsilon = .69$; linear trend: $F(1,15) = 69.4$, $p < .0009$]. There also was a main effect of treatment [$F(3,45) = 7.6$, $p < .001$, GG-$\epsilon = .83$] that was caused by an overall reduction in anxiety with diphenhydramine [$F(1,15) = 17.1$, $p < .001$], low alprazolam [$F(1,15) = 4.8$, $p = .04$], and high alprazolam [$F(1,15) = 14.9$, $p = .002$] treatments compared to placebo (Figure 2). However, unlike the startle results, there was no treatment × condition interaction, indicating no significant difference in subjective

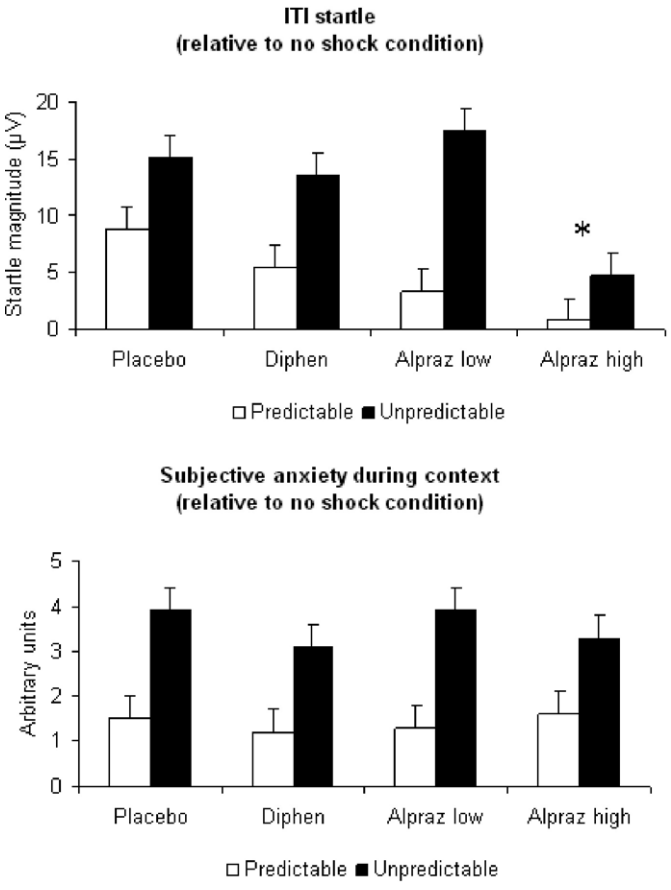

Figure 2. Responses during ITI (context) in each treatment. (**A**) Contextual potentiated startle. Difference scores between startle magnitudes in the threat conditions (predictable and unpredictable) and startle magnitudes in the no-shock condition. *The increased startle from the neutral to the predictable and unpredictable conditions was significantly reduced ($p < .05$) by high alprazolam (alpraz), compared to the other treatments. (**B**) Subjective anxiety. Difference scores between reported anxiety in the threat conditions (predictable and unpredictable) and reported anxiety in the no-shock condition. There was no significant difference among treatments.

Table 2. Mean (SEM) Scores of Subjectively Reported Mental and Physical Sedation

	Mental Sedation		Physical Sedation	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Placebo	10.2 (1.0)	13.6 (1.7)	9.8 (1.1)	13.6 (1.7)
Diphenhydramine	11.0 (1.4)	22.6 (1.9)	0.0 (1.3)	22.7 (1.9)
Alprazolam, low	9.6 (1.0)	17.4 (1.4)	9.7 (.9)	17.4 (1.4)
Alprazolam, high	9.9 (1.2)	20.9 (1.6)	10.6 (1.0)	20.9 (1.6)

anxiety across conditions among the three active treatments (Figure 2B).

Mental and Physical Sedation

The mental and physical sedation data from the mood rating scale are shown in Table 2. Results were analyzed with treatment (placebo, diphenhydramine, alprazolam low, or alprazolam high) × condition (baseline or posttreatment) ANOVAs. For both mental sedation and physical sedation, there were significant main effects of treatment [$F(3,45) = 6.5$, $p < .001$, GG-$\epsilon = .78$ and $F(3,45) = 5.4$, $p < .003$, GG-$\epsilon = .92$, respectively] and of condition [$F(1,15) = 37.5$, $p < .0009$ and $F(1,15) = 44.0$, $p < .0009$, respectively], as well as a significant treatment × condition interaction [$F(3,45) = 8.4$, $p < .001$, GG-$\epsilon = .70$ and $F(3,45) = 9.9$, $p < .0009$, GG-$\epsilon = .79$, respectively]. The interaction reflected the fact that subjects exhibited greater increases in physical and mental sedation from the baseline to the posttreatment period with diphenhydramine, low alprazolam, and high alprazolam compared with placebo (all $p < .05$). There also was a greater increase in mental and physical sedation with the diphenhydramine and high-alprazolam, compared with low-alprazolam, treatments (all $p < .05$). Finally, there was no significant difference in mental and physical sedation between diphenhydramine and high-alprazolam treatments.

Discussion

The present study sought to establish a psychopharmacological distinction by using the benzodiazepine alprazolam between a phasic aversive response to a threat cue (cued fear) and a more sustained aversive response associated with the experimental context in which shocks are anticipated (contextual anxiety). The experimental model to elicit these two aversive states was based on preclinical data in rodents (Davis 1998) and on empirical work in our laboratory that used the threat of predictable (signaled) and unpredictable (unsignaled) shocks (Grillon et al 2004). Consistent with our hypothesis, alprazolam did not affect phasic fear-potentiated startle to the threat cue but reduced the sustained potentiation of startle in the predictable and the unpredictable condition. This effect cannot be attributed to an artifactual sedative effect of alprazolam on baseline startle (Walker and Davis 2002b) for two reasons. First, baseline startle responses, as well as mental and physical sedation, were affected to the same extent by high alprazolam and diphenhydramine, indicating that diphenhydramine was an appropriate nonspecific active control substance. Yet, contextual startle potentiation in the predictable and unpredictable conditions was reduced significantly in the high alprazolam compared to the diphenhydramine treatment. Second, if the reduction in contextual fear-potentiated startle was a result of sedation, it is unclear why such an effect would not have affected fear-potentiated startle to the threat cue. These results suggest that alprazolam exerted a differential effect on potentiated startle to an explicit threat cue and to contextual cues.

It could be argued that the treatment effect of the high dose of alprazolam on ITI startle in the predictable and unpredictable condition was artifactually caused by a floor effect. This explanation is unlikely because the floor effect for startle magnitude is an absence of eyeblink response. In the present study, the magnitude of startle in the high-alprazolam condition was clearly above 0 (about 20 μV). Alternatively, it could be argued that the lack of effect of alprazolam on fear-potentiated startle to the cue in the predictable condition was caused by a ceiling effect. This, again, is unlikely. First, we analyzed the startle habituation data (not shown) and found much larger responses during startle habituation compared response to the threat cues in the predictable condition. For example, the group mean magnitude of the first habituation startle in the placebo condition was 91 μV, which is larger than either the mean response to the first startle in the predictable cue (74 μV) or than the mean startle response to all the cues in the predictable condition (57.9 μV).

The present findings are consistent with preclinical and clinical studies distinguishing cued fear from contextual anxiety. Preclinical studies show that the CeA mediates phasic responses to explicit threat cues and that the BNST mediates sustained responses to contextual stimuli (Walker et al 2003). Clinical investigations have reported normal fear-potentiated startle to an explicit threat cue but elevated contextual fear-potentiated startle in patients with anxiety disorders (Cuthbert et al 2003; Grillon et al 1991, 1997; Grillon and Davis 1997; Pole et al 2003).

The present results also are consistent with animal data showing that benzodiazepines reduce sustained forms of startle potentiation. Walker and Davis (2002a) reported that the light-enhanced startle effect was alleviated by the benzodiazepine chlordiazepoxide. However, the lack of effect of alprazolam on fear-potentiated startle to an explicit threat cue apparently conflicts with cued fear conditioning data in rodents (Hijzen et al 1995; Melia and Davis 1991). One important difference between the present study and preclinical studies is the reliance of animal studies on associative-learning processes such as fear conditioning. The reduced fear-potentiated startle after benzodiazepine administration in animal models that rely on conditioning cannot be attributed unambiguously to an anxiolytic effect as opposed to an effect on learning or memory (Walker and Davis 2002a). In addition, it is possible that the amount of drug used in animals is not comparable to that used in humans. Nevertheless, a recent fear-conditioning study in humans supports our findings. Scaife et al (2005) found that diazepam blocked the acquisition but not the expression of fear-potentiated startle to an explicit cue.

In terms of current results for subjective ratings of anxiety the cue and contextual manipulations induced the same basic pattern of results found in the startle data. In the predictable condition, subjective anxiety was larger in the presence than in the absence of the threat cue. Like the startle data, this increase in subjective anxiety was unaffected by drug treatments. As for the context effect, both startle magnitude and the subjective ratings of anxiety to the context increased linearly from the

no-shock to the predictable to the unpredictable conditions. However, treatment effects on these subjective reports did not follow the startle results; instead of a significant treatment × condition interaction, there was only a main effect of treatment. This indicates that though the subjects were feeling less anxious overall under all three drug treatments compared with placebo, they did not report context-specific reductions in anxiety under alprazolam. Apparently, startle was sensitive to subtle effects of the treatments that were not accessible to subjective measures of anxiety.

One obvious reason for this difference is that whereas startle was used to probe contextual anxiety online, the subjective anxiety measures were retrospective. The passage of time may have obscured subtle differences in responding because of the complexity of the design. The design included six different conditions (three contextual manipulations, each with and without the presence of a cue), for which subjective anxiety was measured retrospectively. The retrospective subjective anxiety data appeared to have been strongly influenced by sedation. This is suggested by the fact that the pattern of retrospective ratings of anxiety exactly paralleled the ratings of mental and physical sedation (Table 1). Future studies may benefit from using an online measure of subjective anxiety.

How could alprazolam reduce contextual anxiety? It is unlikely that the present results were the result of a gross cognitive deficit, such as memory impairment. Subjects did not have to remember the contingency between threat and the different conditions because the instructions were written on a monitor screen throughout the duration of each condition. A purely anxiolytic effect on sustained anxiety could be achieved, for example via action at the level of structures involved in this type of response (i.e., the BNST). Alternatively, alprazolam could reduce anxiety by facilitating distraction from stressful cognitions associated with the threat experiment. Distraction from stressful cognitions may have been more likely to occur in the unpredictable condition when the threat was long lasting, compared to the predictable condition, in which the threat cue signaled an imminent danger, resulting in a reduction in contextual anxiety but not phasic fear. Such a distraction could have been amplified by drowsiness, but this is unlikely given that the sedative drug diphenhydramine did not reduce the enhanced startle in the unpredictable context.

The limitations of the present study must be considered when interpreting the results. It is possible that alprazolam effects on potentiated startle did not depend on the qualitative nature of the induced emotion (e.g., fear vs. anxiety or explicit vs. contextual cues), but on the intensity of the aversive reaction. According to this view, benzodiazepines could reduce potentiated startle to stimuli or situations that elicit little fear or anxiety, such as contextual anxiety, but are ineffective when higher levels of fear or anxiety are involved, such as during a threat cue. Such a possibility should be tested experimentally by examining the effect of benzodiazepines on threat cues that elicit different levels of fear. However, the Scaife et al (2005) fear-conditioning study may shed light on this issue. Startle potentiation to a cue is much greater during a threat of shock experiment than during fear conditioning (Grillon and Davis 1977; Grillon et al 1991). Yet Scaife et al (2005) found that diazepam did not block the expression of cued fear conditioning. Hence, a weak aversive response is not necessarily affected by benzodiazepines. Another limitation is that we used diphenhydramine as an active control condition because of its sedative effect. The subjective data suggest that diphenhydramine was mildly anxiolytic, and animal data suggest that the histaminergic system may be implicated in anxiety (Fish et al 2004; Privou et al 1998). Because benzodiazepines have muscle relaxant properties, a muscle relaxant may have been a more appropriate control drug.

The present results are consistent with the hypothesis of a functional differentiation between phasic startle potentiation to a threat cue and sustained startle potentiation to contextual threat proposed by Davis (1998). Because of the translational nature of startle studies, it is likely that human and animal models will ultimately yield more insight into the neurobiology of cued fear and contextual anxiety. Differentiating between cued fear and contextual anxiety may help us identify the components of aversive states and their relevance to pathologic anxiety. Assuming that contextual anxiety is relevant to pathologic anxiety (Grillon and Morgan 1999; Grillon et al 1998b), experiments in animals and humans that manipulate contextual fear-potentiated startle may be helpful for screening novel anxiolytics.

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