# Effects of Experimental Context and Explicit Threat Cues on Acoustic Startle in Vietnam Veterans with Posttraumatic Stress Disorder

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**Background:** *The hypothesis that exaggerated startle in Vietnam veterans with posttraumatic stress disorder (PTSD) reflects an anxiogenic response to stressful contexts was tested.*

**Methods:** *Thirty-four nonmedicated Vietnam veterans with PTSD, and 17 combat and 14 civilian non-PTSD controls participated in two testing sessions over separate days. Acoustic startle stimuli were delivered alone or in a test of prepulse inhibition. In the first session, startle was assessed without experimental stress. In the second session, startle was investigated during a stressful "threat of shock" experiment, when subjects anticipated the administration of shocks during threat periods and during safe periods when no shocks were anticipated.*

**Results:** *The magnitude of startle did not differ significantly among the three groups in the first session, but was increased throughout the threat of shock experiment in the PTSD veterans in the second session. The actual increase in startle in the threat compared to the safe condition did not significantly differ among the three groups. Prepulse inhibition was reduced in the PTSD veterans, compared to the non-PTSD civilians, but not compared to the non-PTSD veterans.*

**Conclusion:** *Exaggerated startle in Vietnam veterans with PTSD reflects an anxiogenic response to an environment that is experienced as stressful.* Biol Psychiatry 1998; 44:1027–1036 © *1998 Society of Biological Psychiatry*

**Key Words:** PTSD, startle reflex, contextual fear, veterans, stress

## Introduction

Although exaggerated startle is a symptom of posttraumatic stress disorder (PTSD), studies that have assessed the startle reflex in individuals with PTSD have provided conflicting results. Whereas some studies have reported exaggerated baseline startle (Orr et al 1995; Butler et al 1990), others have found a normal (Orr et al 1997; Grillon et al 1996) or reduced response (Ornitz and Pynoos 1989). These discrepancies are not explained by methodological differences between studies, because three studies using the same procedure reported different results (Orr et al 1995, 1997; Shalev et al 1992).

One potential explanation for the above discrepancies involves the nature of experimental contexts. Because startle is potentiated by fear, it is possible that the symptom of "exaggerated startle" reflects an anxiogenic response to an aversive context. Results from several studies at the Connecticut Veterans Administration Medical Center are consistent with this hypothesis. We recently reported normal baseline startle in Vietnam veterans with PTSD in a procedure with little experimental stress (Grillon et al 1996); however, we found exaggerated startle throughout experiments that involved some degree of stress (Morgan et al 1995a, 1995b; Grillon et al in press). For example, in a "fear-potentiated startle" study, startle was investigated during "threat" conditions, when unpleasant electric shocks were anticipated, and during "safe" conditions, when no shocks were anticipated (Morgan et al 1995a). Startle was elevated in the PTSD patients *throughout* the experiment, including the "safe" condition. The finding of normal startle in the absence of stress, but elevated startle under stressful conditions suggests that the symptom of "exaggerated startle" in Vietnam veterans with PTSD is a sign of an enhanced anxiogenic response to a "threatening" context. Of note, in the threat of shock experiment, the response of the PTSD group to the threat signal was similar to that of the control group, suggesting that veterans with PTSD exhibit differential fear responses to explicit (e.g., the threat signal) and contextual (e.g., the experimental room) stimuli. These results are particularly meaningful in the light of recent preclinical findings suggesting the involvement of different brain structures in these two types of fear (Kim and Fanselow 1992; Phillips and LeDoux 1992; Davis et al 1995) (see Discussion).

The hypothesis that Vietnam veterans with PTSD ex-

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Table 1. Mean (SD) Age and Psychometric Scores

| Group | Age (years) | Mississippi | CES[a] | Trait anxiety | State anxiety Session 1 | State anxiety Session 2 |
|---|---|---|---|---|---|---|
| PTSD veterans ($n$ = 34) | 46.0 | 128.0 | 27.5 | 57.7 | 50.8 | 52.2 |
| | (3.4) | (20.3)[b] | (8.9)[c] | (10.4)[d] | (12.6)[d] | (11.7)[d] |
| Combat controls ($n$ = 17) | 42.2 | 70.2 | 21.7 | 34.9 | 31.8 | 34.3 |
| | (4.8) | (17.0) | (9.3) | (10.8) | (8.2) | (6.4) |
| Civilian controls ($n$ = 14) | 44.5 | — | — | 30.0 | 27.7 | 29.2 |
| | (3.9) | | | (5.2) | (5.4) | (2.6) |

[a]Combat exposure scale.
[b]$p < .0009$ relative to combat controls.
[c]$p < .03$ relative to combat controls.
[d]$p < .0009$ relative to combat and civilian controls.

hibit exaggerated startle in stressful contexts but not at baseline would draw stronger support if this effect was demonstrated in a within-subjects design, where the same individual would have normal startle in the absence of stress, but exaggerated startle during a stressful procedure. The present study investigated this possibility by examining startle in two experimental sessions separated by 4–5 days. The first session, which was a test of baseline startle, had a relatively low level of stress. In contrast, the level of stress was greatly increased in the second session by performing a fear-potentiated startle experiment that involved the anticipation of unpleasant electric shocks. The basic design was similar to the one used in Morgan et al (1995a) (i.e., shocks were anticipated during threat conditions, but not during safe conditions). In such an experiment, the phasic state of fear induced by the specific threat condition may be considered as being superimposed on a generally anxious state caused by the ambient threat of participating in a stressful experiment (Breznitz 1967; Grillon et al in press). Thus, two types of fear were assessed: ambient fear related to the experimental context (e.g., experimental room, shock electrodes) and fear specifically caused by the imminent anticipation of shocks during the threat condition. Based on our previous studies (Grillon et al 1996; Morgan et al 1995a), it was hypothesized that there would not be differences among groups in baseline startle in the first session 1 and in startle potentiation by the threat signal in session 2; however, due to their hypothesized increased sensitivity to contextual fear, the veterans with PTSD were expected to show greater startle in session 2, compared to the non-PTSD subjects, before and especially after the placement of the shock electrodes.

A second aim of the study was to examine prepulse inhibition (PPI). PPI refers to the ability of a weak prepulse to reduce the startle response to a subsequent startle-eliciting stimulus. Animal and human studies indicate that PPI can be affected by stress, but the nature of the change is still unclear. PPI is reduced in the rat following immersion in cold water (Leitner 1986) and can be increased or reduced in humans during a threat of shock experiment (Grillon and Davis 1997a). PPI deficits in humans have been associated with perceptual abnormalities and deficiencies in gating irrelevant thoughts (Braff and Geyer 1990). Reduced PPI in PTSD has been reported in some studies (Ornitz and Pynoos 1989) but not others (Butler et al 1990). Grillon et al (1996) reported that PPI was reduced in veterans with PTSD, compared to non-PTSD civilians, but not compared to the non-PTSD veterans. The present experiment further investigated this question by examining the impact of fear on PPI in veterans with PTSD.

## Methods and Materials

### *Subjects*

Subjects were 34 inpatient Vietnam combat veterans with PTSD free of medication for at least 1 month. Comparison subjects consisted of 17 healthy Vietnam combat veterans without PTSD and 14 healthy civilians. The age of patients and controls did not differ significantly (Table 1). The subjects were paid for their participation in the study. They all gave written and informed consent. None had participated in any of the previous psychophysiological experiments.

All patients met criteria for PTSD per DSM-III-R, Structured Clinical Interview for DSM-III-R (SCID), and a consensus diagnosis team. Patients with a major medical illness, organic brain syndrome, or schizophrenia were excluded from the study. Patients also were evaluated with the SCID for comorbid psychiatric diagnoses. Current comorbid diagnoses were bipolar disorder ($n$ = 1), major depressive disorder ($n$ = 17), substance abuse/dependence ($n$ = 4), panic disorder with and without agoraphobia ($n$ = 6 and $n$ = 4, respectively), social phobia ($n$ = 8), obsessive–compulsive disorder ($n$ = 1), and antisocial behavior/borderline personality ($n$ = 1). Control subjects had no current major medical illnesses or psychiatric disorders based on SCID–nonpatient criteria. Toxicology screening confirmed that all subjects were free of psychoactive drugs or illicit substances for at least 4 weeks prior to testing. Subjects with hearing deficits

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Table 2. Experimental Design

| Session | Period/Imminent description | # of blocks[a] | Shock electrodes | Threat/safe lights on | Ambient threat | Threat |
|---|---|---|---|---|---|---|
| 1 | 1. Initial baseline startle testing, no shock electrodes, no ambient threat | 4 | No | No | No | No |
| 2 | 1. Second baseline startle testing, shock electrodes off, with ambient threat | 4 | No | No | Yes | No |
| 2 | 2. First startle testing, with shock electrodes on, safe–threat signals off | 4 | Yes | No | Yes | No |
| 2 | 3. First fear-potentiated startle test: safe and threat conditions[b] | 3 | Yes | Yes | Yes | No |
| | | 3 | Yes | Yes | Yes | Yes |
| 2 | Rest period/no startle stimuli | | | | | |
| 2 | 4. Second startle testing, with shock electrodes on, safe–threat signals off | 4 | Yes | No | Yes | No |
| 2 | 5. Second fear-potentiated startle test: safe and threat conditions[b] | 3 | Yes | Yes | Yes | No |
| | | 3 | Yes | Yes | Yes | Yes |

[a]Each block consisted of each of the four trial types (i.e., 98-dB pulse-alone, 103-dB pulse-alone, 70-dB prepulse + 98-dB pulse, 70-dB prepulse + 103-dB pulse).
[b]The safe and threat lights were presented alternatively three times each in each fear-potentiated startle test.

in the 500–3000-Hz range during audiologic screening with a Welch Allyn device were excluded from the study. About 1/3 of the veterans with and without PTSD had hearing loss at 4000 Hz; however, there was no difference in startle responsivity or PPI in veterans with and without such a deficit. Combat history was verified by military discharge forms. Veterans also completed the Mississippi scale for combat-related PTSD (Keane et al 1988) and the Combat Exposure Scale (CES) (Lund et al 1984).

## *Stimuli and Apparatus*

Recordings took place in a sound-attenuated chamber at the Connecticut Veterans Administration Medical Center. The delivery of the acoustic startle stimuli and the recording of the eyeblink response were controlled by a commercial startle system (San Diego Instrument). The acoustic startle stimuli consisted of 40-msec duration bursts of white noise with an instantaneous rise-time at intensities of 98 and 103 dB(SPL) presented binaurally through headphones (Telephonics model TDH-39P). There was no background sound. The sounds were calibrated with a Quest Electronics device (model 215). The startle stimuli were delivered alone (pulse-alone) or preceded by a 30-msec 70-dB(SPL) white noise prepulse (prepulse + pulse) with an onset asynchrony of 120 msec. Thus, four different types of trials were used: 98-dB pulse-alone, 103-dB pulse-alone, 70-dB prepulse followed by a 98-dB pulse, and 70-dB prepulse followed by a 103-dB pulse.

The eyeblink reflex was measured with two disk electrodes (Ag-AgCl) below the left eye (impedance <5 kΩ). The ground electrode was placed on the left arm. Electromyographic activity was filtered (1–500 Hz) and digitized at 1 kHz for 250 msec from the onset of the acoustic stimuli.

The electric shock (1.5 mA, 5-msec duration) was generated by a Constant Current Unit (Grass Inst.) delivered through two pure tin disk electrodes placed on the left wrist.

## *Design*

The study was designed to examine fear induced by 1) an experimental context (e.g., the ambient threat of participating in an experiment involving the administration of shocks); and 2) an explicitly threatening signal (e.g., imminent threat). There were two sessions separated by 4–5 days (Table 2; see also Figure 1). Shocks were anticipated and administered in session 2, but not in session 1, making the level of ambient threat greater in session 2, compared to session 1. Session 1 assessed baseline startle during a single period (session 1/initial baseline startle testing/no ambient threat). Session 2 consisted of five separate periods, two of which had alternating safe and threat conditions (first and second fear-potentiated startle tests). During the fear-potentiated startle tests, shocks could be administered during threat but not during safe conditions that were signaled by colored lights (i.e., blue and green). Thus, in session 2 startle was recorded 1) before the shock electrodes were attached on the participants' wrist (second baseline startle testing/shock electrodes off/with ambient threat); 2) after the placement of the shock electrodes, but before the threat and safe conditions were started (startle testing with shock electrodes on/safe–threat signals off); 3) during threat and safe conditions (first fear-potentiated startle test); and following a short break, during a repetition of 2) and 3) above, that is, during 4) a second startle testing with shock electrodes on and safe–threat signals off; and 5) a second fear-potentiated startle test. Participants received one or two shocks. The shocks were

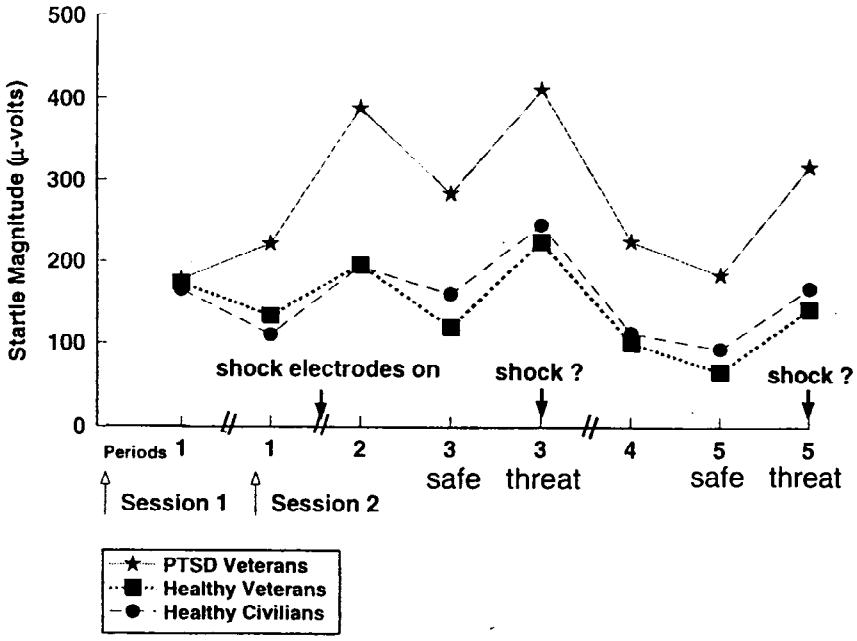


Figure 1. Mean magnitude of startle to pulse-alone trials (averaged over blocks and intensities) during each period. The // signs indicate that the periods were not continuous.

delivered following the second fear-potentiated startle test after all the startle data had been collected (see below).

The reason for assessing startle before and immediately after the shock electrodes were attached in session 2 was because the placement of shock electrodes raises the level of anxiety and potentiates startle, even before subjects are actually threatened with a shock (Grillon and Ameli in press).

Sixteen startle trials were presented during 1) the initial baseline startle testing (session 1); 2) the second baseline startle testing (session 2); 3) the first startle testing with shock electrodes on (safe–threat signals off); and 4) the second startle testing with shock electrodes on (safe–threat signals off). They were delivered in four blocks with each block containing the four types of trials (98-dB pulse-alone, 103-dB pulse-alone, 70-dB prepulse + 98-dB pulse, 70-dB prepulse + 103-dB pulse) in a prearranged order, counterbalanced between groups. The intertrial intervals varied from 17 to 23 sec. The first fear-potentiated startle test consisted of three alternating threat and safe conditions that were signaled by the blue and green colored lights (60-W bulbs) located 2 m in front of the subjects. Each threat/safe condition lasted approximately 80 sec. The order of presentation of the threat and safe conditions and the association between colored lights and conditions were counterbalanced across subjects. During each threat and each safe condition, the four types of trials (two pulse-alone trials, two prepulse trials) were also presented in a prearranged order counterbalanced between groups. The second fear-potentiated startle test was similar to the first one.

Fear to the explicit cue (i.e., imminent threat) was operationally defined as the potentiation of startle magnitude from the safe to the threat condition during the first and second fear-potentiated startle tests. Contextual fear was defined as the fear elicited by the ambient threat of session 2. It was examined by comparing the change in startle magnitude 1) from session 1 to session 2 (initial baseline startle testing/no ambient threat vs. second baseline startle testing/with ambient threat); and 2) in session 2, from the second baseline startle testing (no shock electrodes) to the first startle testing with shock electrodes on (safe–threat signals off). One of the distinctions that was made between fear to explicit and contextual cues was that participants were in actual danger of receiving shocks during explicit threat cue testing but not during contextual fear testing.

## *Procedure*

During the initial recruitment, participants were informed that the study examined their reactivity to loud sounds under various stress conditions in two separate sessions. They were told that unpleasant electric shocks would be administered during the second session, but not during the first one. After the arrival of the participants in the laboratory in session 1, they were reminded that no shock would be administered. When subjects arrived for the second session, there were told that their reactivity to loud sounds would first be tested as in the first session, and that this portion of the testing did not involve the placement of shock electrodes or the administration of unpleasant shocks (session 2/second baseline startle testing). Approximately 2 min after the second baseline startle testing period, the shock electrodes were attached on the subjects' wrist. Participants were then informed that the threat of shock part of the study was starting (session 2/first startle testing with shock electrodes on). They were told that 1) unpleasant electric shocks could be delivered at any time when the threat light (i.e., blue light), but not the safe light (i.e., green light), was turned on; and 2) they would receive between one and three shocks. Finally, they were told that initially startle stimuli would be presented with the safe and threat lights turned off and no shocks could be administered (first startle testing with shock electrodes on with safe–threat signals off).

Participants were given a 5-min break after the first fear-

potentiated startle test (the shock electrodes were left on their wrist). A second startle testing with shock electrodes on and safe–threat light off followed by a second fear-potentiated startle test was then performed. No shocks were administered during the first fear-potentiated startle test. The shock(s) was (were) administered at the end of the second fear-potentiated startle test, 5 sec following the last startle trial if the last condition was a threat light. If the last condition was a safe light, the threat light was turned on for an additional 20 sec, and one shock was administered. In about 1/3 of the subjects, an additional safe and threat condition was added, and another shock was administered. The data during this additional period were not analyzed. This procedure was implemented to prevent potential subjects from learning about the number of shocks.

Subjects were given the state portion of the State–Trait Anxiety Inventory (STAI) (Spielberger 1983) upon arrival in the laboratory in each session. They were also given the trait portion of the STAI following the end of session 1.

### *Data Reduction and Data Analysis*

The method to analyze the eyeblink is presented in detail elsewhere (Grillon et al 1991). Briefly, following digital filtering of the electromyographic (EMG) signal with a 20.9-Hz low-pass filter, the eyeblink data were analyzed using a program derived from Balaban et al (1986). Peak amplitude was determined in the 21–100 ms following stimulus onset. It was expressed relative to EMG activity during a 20-msec period following stimulus onset. A zero response score was given if no response was detectable. Zero startle scores were included in the data analysis (magnitude scores). Trials were rejected if the baseline EMG activity was unstable or onset eyeblink occurred within 20 msec following probe onset. The number of discarded trials was low and did not significantly differ among groups (1.6%, 1.4%, and 1.3% in the non-PTSD civilians, non-PTSD veterans, and PTSD veterans, respectively).

Preliminary analyses of the data indicated that the magnitude of startle and prepulse inhibition did not habituate differentially within blocks in the three groups. Similarly, the intensity of the startle stimulus did not affect the groups differentially. To simplify the presentation of the data, the magnitude measures for eyeblinks to pulse-alone and prepulse + pulse trials were averaged separately across blocks and stimulus intensities within each period. Startle magnitudes to pulse-alone trials were analyzed separately from prepulse and pulse trials. PPI was expressed as a percent change from the magnitude of startle to pulse-alone to the magnitude of startle to prepulse + pulse trials ([pulse-alone minus prepulse + pulse)/(pulse-alone)] × 100).

The data were analyzed with mixed model analyses of variance (ANOVAs) with repeated measures and Pearson correlations. To examine contextual fear, startle magnitude to pulse-alone in the four periods when subjects were not actually threatened by the shock (i.e., initial baseline startle testing, second baseline startle testing, first startle testing with shock electrodes on, second startle testing with shock electrodes on) were entered into a two-way ANOVA [period (4) × group (3)]. Fear-potentiated startle was analyzed by entering the data of the first and the second fear-potentiated startle tests in a three-way ANOVA [condition (safe, threat) × fear-potentiated startle test (first, second) × group (3)].

Similar analyses were performed on the PPI data. Several participants had no identifiable startle/eyeblink responses to all pulse-alone trials in one of the periods, especially in the second safe condition period. Because prepulse inhibition cannot be assessed from a zero-amplitude startle response, only data from session 1, and from the second baseline startle testing, first startle testing with shock electrodes on, and first fear-potentiated startle test were included in the PPI analysis. Thus, two types of analyses were performed: 1) a two-way ANOVA [period (initial baseline startle testing/session 1, second baseline startle testing/session 2, first startle testing with shock electrodes on/session 2) × group (3)]; and 2) a two-way ANOVA [condition (safe, threat) × group (3)] using the data of the first fear-potentiated startle test.

To reduce the number of statistical tests, the post hoc analyses contrasted 1) the PTSD veterans to the non-PTSD veterans; and 2) the PTSD veterans to the non-PTSD civilians to assess the specificity of the findings.

Analyses of covariance were also used to examine whether differences between veterans with and without PTSD were due to differences in exposure to combat. The covariate was the CES score. Reduced degrees of freedom (Greenhouse–Geisser) were employed to counter violations of the sphericity assumption underlying ANOVA with repeated measures.

## Results

### *Psychometric Measures*

The Mississippi, CES, and trait anxiety scores (Table 1) were significantly greater in the veterans with PTSD, compared to the combat controls [$t(49) = 10.9, p < .001$; $t(49) = 2.1, p < .03$; $t(49) = 7.1, p < .001$, respectively]. Trait anxiety was greater in the veterans with PTSD, compared to the civilian controls [$t(49) = 9.3, p < .001$]. State anxiety scores were analyzed using a two-way ANOVA with group (PTSD, combat controls, civilian controls) and session (1, 2) as factors. The group main effect was significant [$F(2,62) = 46.0, p < .0009$], reflecting the fact that state anxiety was greater in the veterans with PTSD, compared to the combat [$F(1,62) = 42.4, p < .0009$] and civilian [$F(1,62) = 56.7, p < .0009$] controls. There was a trend for state anxiety to increase from the first to the second session [$F(2,62) = 2.7, p < .1$]. This effect did not differ significantly among groups.

### *Startle to Pulse-Alone Trials*

There were subjects with small startle responses (see below) during session 1; however, all subjects were included in the analysis because of the possibility that startle "nonresponders" might become responders in the more stressful session 2.

Figure 1 presents the magnitude of startle during each

Table 3. Mean (SE) Percent Prepulse Inhibition (PPI) in the Three Groups

| | Session | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | | | |
| | | | Period | | |
| | 1 | 1 | 2 | 3S | 3T |
| PTSD veterans[a] | 64.6 (6.1) | 71.1 (3.1) | 61.8 (3.3) | 76.7 (4.5) | 82.7 (2.6) |
| Combat controls | 81.2 (7.8) | 79.3 (9.4) | 57.6 (6.8) | 78.2 (9.4) | 88.4 (6.8) |
| Civilian controls | 92.5 (3.7) | 93.2 (1.9) | 66.4 (6.8) | 89.1 (3.4) | 95.8 (7.3) |

S, safe; T, threat.
[a] $p < .007$ compared to non-PTSD civilians.

period, that is during the: 1) initial baseline startle testing (session 1/period 1); 2) second baseline startle testing (session 2/period 1); 3) first startle testing with shock electrodes on and safe–threat signals off (session 2/period 2); 4) first fear-potentiated startle test (session 2/period 3); 5) second startle testing with shock electrodes on and safe–threat signals off (session 2/period 4), and 6) second fear-potentiated startle test (session 2/period 5).

The pattern of startle reactivity in session 1 and session 2 differed among groups [group × period: $F(6,186) = 3.6$, $p < .003$, $\epsilon = .94$]. Startle magnitude did not significantly differ among groups in session 1 [$F(2,62) = 0.03$], but was significantly greater in session 2 in the PTSD veterans, compared to the non-PTSD veterans [$F(1,62) = 6.5$, $p < .01$] and civilians [$F(1,62) = 5.4$, $p < .02$]. Post hoc analyses based on our a priori hypotheses indicated that this effect was due to the combination of two factors. First, startle magnitude was differently affected by the experimental context (participation in an experiment where no shocks are administered—initial baseline startle testing/session 1/period 1—versus participation in an experiment where shocks will be administered—second baseline startle testing/session 2/period 1) in PTSD veterans, compared to non-PTSD veterans [$F(1,62) = 5.6$, $p < .02$] and civilians [$F(1,62) = 3.5$, $p < .06$]. Second, although the placement of the shock electrodes in session 2 increased startle (first startle testing with shock electrodes on and safe–threat signals off—period 2) in the PTSD veterans [$F(1,62) = 46.1$, $p < .001$], non-PTSD veterans [$F(1,62) = 12.7$, $p < .001$], and non-PTSD civilians [$F(1,62) = 8.4$, $p < .005$], the magnitude of this increase was greater or tended to be greater in the PTSD veterans, compared to the non-PTSD veterans [$F(1,62) = 3.3$, $p < .07$] and civilians [$F(1,62) = 4.6$, $p < .04$].

To examine whether differences in combat exposure could explain these findings, the data were reanalyzed using only the two veterans groups and the CES scores as a covariate. Similar results were obtained. The magnitude of startle was differently affected by the two sessions in the two groups [$F(3,144) = 4.1$, $p < .008$, $\epsilon = .92$]. Startle magnitude was significantly elevated in the PTSD veterans, compared to the non-PTSD veterans in session 2 [$F(1,48) = 7.6$, $p < .008$], but not in session 1 [$F(1,48) = 0.3$].

Fear response to the explicit threat signal was assessed by comparing the magnitude of startle in the threat and in the safe conditions during the fear-potentiated startle tests (periods 3 and 5). As expected, startle was significantly potentiated during shock anticipation [fear-potentiated startle: condition: $F(1,62) = 50.6$, $p < .001$]. The magnitude of this potentiation did not differ significantly among groups [group × period: $F(2,62) = 1.5$].

### *Prepulse Inhibition*

Seven participants (2 veterans with PTSD, 3 veterans without PTSD, and 2 civilians without PTSD) were excluded from the analysis because they had a zero mean startle amplitude to pulse-alone trials in one or more of the periods that were used in the PPI analysis.

The PPI scores are presented in Table 3. Analysis of PPI during the different periods revealed significant main group [$F(2,55) = 3.4$, $p < .04$] and period [$F(2,110) = 12.8$, $p < .0001$, $\epsilon = .94$] effects, but no significant group × period interaction [$F(4,110) = 2.1$]. Post hoc tests indicated that PPI was significantly smaller in the PTSD veterans, compared to the non-PTSD civilians [$F(1,55) = 6.4$, $p < .01$], but not compared to the non-PTSD veterans [$F(1,55) = 1.0$].

PPI during the fear-potentiated startle test did not significantly differ among groups [$F(1,56) = 0.00$], but was increased in the threat, compared to the safe condition [$F(1,56) = 6.9$, $p < .01$].

### *Correlations*

Pearson correlations were performed between the Mississippi, CES, and trait and state anxiety scores, and startle in the periods when group differences emerged, that is, in session 2 during the second baseline startle testing (period 1) and first startle testing with shock electrodes on (period 2). Two types of startle measures were used: the raw

magnitude scores and the change or difference scores from the first to the second session. The Mississippi scores showed small significant correlations with the difference startle scores but not with the raw startle magnitude scores. The Mississippi scores correlated positively with the changes in startle magnitude from session 1 to session 2 before ($r = .27, p < .05$) and after ($r = .33, p < .01$) the shock electrodes were placed on the participants' wrist. Pearson correlation between the psychometric and PPI scores were not significant.

### *Comorbidity*

A large number (50%) of veterans with PTSD had comorbid major depression. Statistical analyses of data in veterans with PTSD with and without comorbid major depressive disorder did not reveal any significant difference in these two groups.

## Discussion

The magnitude of startle was similar among PTSD and non-PTSD participants during session 1, when no shocks were anticipated. In contrast, startle was elevated in the PTSD group throughout the more stressful session 2, when the experiment involved the administration of shocks. Of note, the veterans with PTSD exhibited "exaggerated" startle when they were not at risk of receiving a shock, even when the shock electrodes were not in place (session 2/period 1). In addition, despite this elevation in overall startle level in the PTSD veterans during session 2, the magnitude of fear-potentiated startle during the specific threat condition did not differ significantly from that of the comparison group. These results suggest that the PTSD veterans had an abnormal emotional response to the ambient threat of the experimental context, but a normal fear response to the explicit threat cue. Such findings provide potential insights into psychological and neurophysiological mechanisms that may contribute to exaggerated startle in Vietnam veterans with PTSD.

The enhanced magnitude of startle in the PTSD veterans in session 2 came from two sources: simple participation in a stressful experiment and placement of the shock electrodes. Participation in an experiment where unpleasant electric shocks were anticipated potentiated startle in the PTSD group, even when shock electrodes were not attached and when there was no actual danger of receiving a shock. This was reflected by an increase in the magnitude of startle from session 1 to session 2 (initial baseline startle testing versus second baseline startle testing), which contrasted with the reduction found in the two comparison groups. Attaching the shock electrodes produced an upward shift in baseline startle levels in all three groups, but this effect was greater in the PTSD, compared to the comparison groups. These results suggest an abnormally elevated anxiogenic response to contextual stimuli in the veterans with PTSD.

Of note, the stressful environment in this study was not a situational reminder of the trauma. This distinguishes the present results from previous findings showing a elevated conditioned emotional response to trauma-related stimuli in Vietnam veterans with PTSD (Orr et al 1993; Pallmeyer et al 1986; Malloy et al 1983), and indicates that PTSD is associated with deficits in multiple affective response systems.

Although the Vietnam veterans with PTSD exhibited an abnormal emotional response to the experimental context, their affective response to impending aversive events (i.e., threat conditions) was normal. Similar results were found previously (Morgan et al 1995a). It could be argued that because the level of startle reactivity was elevated in the PTSD group, the magnitude of the eyeblink response during the threat condition reached a ceiling level that prevented accurate assessment of the effect of shock anticipation on startle. This explanation seems unlikely, because startle levels higher than those in the threat condition were obtained in the first block of the first fear-potentiated startle test (not shown in Figure 1) following the attachment of the shock electrodes (second baseline startle testing with shock electrodes on). In addition, the magnitude of fear-potentiated startle was still not significantly different among PTSD and non-PTSD participants in the second threat conditions, after the baseline startle level had moved downward in the PTSD veterans.

Animal studies suggest that different neurobiological mechanisms are involved in fear to explicit (e.g., a light that has previously been repeatedly associated with a shock) and contextual (e.g., the cage where the experiment took place) stimuli. Amygdala lesions suppress both explicit and contextual fear conditioning, whereas lesions of the hippocampus (Kim and Fanselow 1992; Phillips and LeDoux 1992) or bed nucleus of the stria terminalis (BNST) (McNish et al 1996) suppress only contextual fear conditioning. These data suggest that the hippocampus and BNST may be especially important in contextual fear, compared to explicit cue conditioning.

A similar dissociation among these limbic structures may also apply to the effect of stress hormones, such as corticotropin-releasing hormone (CRH). CRH produces a constellation of anxietylike behavioral effects after central administration, including a long-lasting increase in startle (Liang et al 1992; Swerdlow et al 1986), which is reduced by the anxiolytic compound chlordiazepoxide (Swerdlow et al 1986). This CRH-induced startle facilitation is blocked by chemical lesions of either the hippocampus or

the BNST, but not by chemical lesions of the amygdala (Lee and Davis 1992). Furthermore, local infusion of CRH antagonist into the BNST, but not the amygdala, blocks the excitatory effect of CRH on startle. These data implicate the hippocampus and BNST, but not the amygdala, in the anxiogenic effects of CRH on startle.

If the brain structures that are associated with fear response to explicit and contextual stimuli are the same in animal and humans, the present results of normal fear response to the explicit threat cue and abnormal contextual fear in the PTSD veterans suggest an involvement of the BNST and/or hippocampus, but not of the amygdala, in Vietnam veterans with PTSD. One possible interpretation of the present findings based on recent data (Lee and Davis 1995) is that Vietnam veterans with PTSD showed heightened CRH responses to the stressful context, leading to an overactivation of the BNST, which in turn increased baseline startle. This hypothesis is consistent with preclinical data indicating sensitized hypothalamic–pituitary–adrenal responses to a novel stressor following chronic stress (Servatius et al 1994; Mason et al 1968).

Needless to say, stimuli that lead to contextual fear in humans and animals differ. Animals learn to associate contextual cues with the aversive stimulus as the experiment develops. The present study suggests that in Vietnam veterans with PTSD, some of these associations were already present upon arrival to the laboratory because of the subjects' knowledge that the experiment involved the administration of shocks. Shock electrodes also constitute powerful contextual cues for both patients and controls, but seem to induce more anxiety in the patients.

We have recently found that Gulf War veterans with PTSD exhibit enhanced contextual fear conditioning, compared to non-PTSD veterans (Grillon and Morgan in submission). The veterans participated in an aversive conditioning procedure that was performed twice over separate days. Baseline startle assessed before the conditioning procedure was reduced from the first to the second testing session in the non-PTSD veterans, whereas it was increased in the PTSD veterans. The increased startle response in the aversive context of the second session is similar to the contextual fear conditioning exhibited by animals when they are placed back in a cage where they have been previously shocked (Blanchard and Blanchard 1972) and by healthy human subjects who were shocked in an unpredictable manner during the first session (Grillon and Davis 1997b). These results confirm that veterans with PTSD are overly sensitive to contextual information. They further suggest that this sensitivity is independent of the chronicity of the disorder.

Various findings concerning PPI were replicated in this study. Consistent with a recent study (Grillon et al 1996), PPI was reduced in the veterans with PTSD, compared to the non-PTSD civilians, but not compared to the non-PTSD veterans. Butler et al (1990) also found no differences in PPI between Vietnam veterans with and without PTSD; however, in a recent study, we have not found PPI deficits in women with sexual assault-related PTSD (Morgan et al 1997). These findings raise the possibility that it is not PTSD per se or exposure to the trauma that cause PPI deficits in veterans with PTSD. We have suggested elsewhere (Grillon et al 1996) that exposure to the intense sounds of gunfire during training and combat could have induced subtle hearing impairment that could affect the efficiency of the prepulse at reducing the magnitude of startle. Subtle hearing impairment would not have been detected by our audiologic screening. This implies that the symptom of exaggerated startle in PTSD does not result from a dysfunction of the inhibitory pathway that modifies startle reactivity.

The increase in PPI during the threat condition replicates the finding of a previous study (Grillon and Davis 1997a). This effect was hypothesized to result from enhanced attention to the environment during shock anticipation. According to this view, enhanced nonspecific attention facilitates information processing, including the processing of the prepulse, which becomes more efficient at inhibiting startle.

Three notes of cautions should be made. First, the design of the study followed a fixed time structure. Hence, the effect in each period might have been dependent on the effects in the preceding periods. In particular, the greater startle in session 2 in the PTSD, compared to the non-PTSD participants could be construed as reflecting a deficit in long-term habituation of startle. Although this could be in itself a significant finding, it is highly unlikely, because the same "exaggerated" startle in a stressful context has been reported previously in studies that did not include a prior baseline startle testing (e.g., no session 1) (Morgan et al 1995a, 1995b; Grillon et al in press). Second, the PTSD veterans had greater CES scores, compared to the combat controls. The findings remained the same after controlling statistically for differences in CES scores, but combat exposure remains a potential confounding factor. Third, although the presence of comorbid major depressive disorder in the veterans with PTSD did not affect the results, it is still unknown to what extent the startle results are specific to PTSD because of the prevalence of other comorbid psychiatric disorders with PTSD. Comorbidity is a part of the PTSD presentation. To seek a group of PTSD patients without comorbidity would be to examine a very nonrepresentative subgroup of PTSD, a biased sample. Abnormalities in contextual fear may, in fact, not be restricted to PTSD. An increase in startle magnitude throughout a threat of shock experiment has been reported in the youngest (<40 years

old) of a group of patients with panic disorder (Grillon et al 1994); however, the same study showed that patients with panic disorder whose age matched that of the veterans with PTSD in the present study did not have elevated startle.

To summarize, Vietnam veterans with PTSD exhibited normal baseline startle in the absence of stress, increased startle throughout a stressful procedure, and normal fear-potentiated startle to an explicit threat signal, suggesting that individuals with chronic PTSD are abnormally sensitive to contextual information, but exhibit appropriate fear to impending danger. The increased sensitivity of veterans with PTSD to stressful context could either be a consequence of exposure to trauma, which could, for example, lower the threshold for emotional reactivity, or reflect a premorbid risk factor for PTSD. These results provide some clues as to the direction of future research. First, the hypothesis that chronic PTSD is associated with increased sensitivity to contextual stimuli should be tested by developing new human models of contextual fear and by examining subjects with PTSD with these models. Second, animal studies should further elucidate the neurobiological substrates of anxiety to contextual stimuli. Preclinical studies suggest a role for the amygdala, the hippocampus, the BNST, and the stress hormone CRH. If these investigations demonstrate that certain classes of drugs are effective in relieving anxiety produced by contextual stimuli, more effective pharmacologic treatments of PTSD could be developed.

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## References

Balaban MT, Losito B, Simons RF, Graham FK (1986): Offline latency and amplitude scoring of the human reflex eyeblink with Fortran IV (computer program abstract). *Psychophysiology* 23:612.

Blanchard DC, Blanchard RJ (1972): Innate and conditioned reactions to threat in rats with amygdaloid lesions. *J Comp Physiol Psychol* 81:281–290.

Braff DL, Geyer MA (1990): Sensorimotor gating and schizophrenia: Human and animal model studies. *Arch Gen Psychiatry* 47:181–188.

Breznitz S (1967): Incubation of threat: Duration of anticipation and false alarm as determinants of the fear reaction to an unavoidable frightening event. *J Exp Res Pers* 2:173–179.

Butler RW, Braff DL, Rausch JL, Jenkins MA, Sprock J, Geyer MA (1990): Physiological evidence of exaggerated startle response in a subgroup of Vietnam veterans with combat-related PTSD. *Am J Psychiatry* 147:1308–1312.

Davis M, Gewirtz JC, McNish KA, Kim M (1995): The roles of the amygdala and bed nucleus of the stria terminalis (BNST) in the acquisition of fear potentiated startle using both explicit and contextual cues. *Soc Neurosci Abs* 21:1224.

Grillon C, Ameli R (in press): Effects of threat of shock, shock electrode placement, and darkness on startle. *Int J Psychophysiol.*

Grillon C, Davis M (1997a): Effects of stress and shock anticipation on prepulse inhibition and the startle reflex. *Psychophysiology* 34:511–517.

Grillon C, Davis M (1997b): Fear-potentiated startle conditioning in humans: Explicit and contextual cue conditioning following paired versus unpaired training. *Psychophysiology* 34:451–458.

Grillon C, Ameli R, Woods SW, Merikangas K, Davis M (1991): Fear-potentiated startle in humans: Effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology* 28: 588–595.

Grillon C, Ameli R, Goddard A, Woods S, Davis M (1994): Baseline and fear-potentiated startle in panic disorder patients. *Biol Psychiatry* 35:431–439.

Grillon C, Morgan CA III, Southwick SM, Davis M, Charney DS (1996): Baseline startle amplitude and prepulse inhibition in Vietnam veterans with PTSD. *Psychiatry Res* 64:169–178.

Grillon C, Morgan CA III, Davis M, Southwick SM (in press): Effect of darkness on acoustic startle in Vietnam veterans with PTSD. *Am J Psychiatry.*

Grillon C, Morgan CA III (submitted): Fear-potentiated startle conditioning to explicit and contextual cues in Gulf war veterans with posttraumatic stress disorder.

Keane TM, Caddell JM, Taylor KL (1988): Mississippi scale for combat-related posttraumatic stress disorder: Three studies in reliability and validity. *J Consult Clin Psychol* 56:85–90.

Kim JJ, Fanselow MS (1992): Modality-specific retrograde amnesia of fear. *Science* 256:675–677.

Lee Y, Davis M (1992): The role of the bed nucleus of the stria terminalis in CRH-enhanced startle: An animal model of anxiety. *Soc Neurosci Abs* 22:465.

Lee Y, Davis M (1995): Role of the bed nucleus of the stria terminalis and the amygdala in the excitatory effect of CRH on the acoustic startle reflex. *Soc Neurosci Abs* 21:1697.

Leitner DS (1986): Alterations in other sensory modalities accompanying stress analgesia as measured by startle reflex modification. *Ann NY Acad Sci* 467:82–92.

Liang KC, Melia KR, Miserendino MJD, Falls WA, Campeau S, Davis M (1992): Corticotropin-releasing factor: Long-lasting facilitation of the acoustic startle reflex. *J Neurosci* 12:2303–2312.

Lund M, Foy D, Sipprelle C, Strachan A (1984): The combat exposure scale: A systematic assessment of trauma in the Vietnam War. *J Clin Psychol* 40:1323–1328.

Malloy PF, Fairbank JA, Keane TM (1983): Validation of a multimethod assessment of posttraumatic stress disorders in Vietnam veterans. *J Consult Clin Psychol* 51:488–494.

Mason JW, Brady JV, Tolliver GA (1968): Plasma and urinary 17-hydroxycorticosteroid responses to 72-hr avoidance sessions in the monkey. *Psychosom Med* 30:608–630.

McNish KA, Gewirtz JC, Davis M (1996): Lesions of the dorsal hippocampus or bed nucleus of the stria terminalis (BNST)

block freezing but not enhancement of the startle reflex in a context previously paired with shock. *Soc Neurosci Abst* 22:1381.

Morgan CA III, Grillon C, Southwick SM, Davis M, Charney DS (1995a): Fear-potentiated startle in post traumatic stress disorder. *Biol Psychiatry* 38:378–385.

Morgan CA III, Grillon C, Southwick SM, Nagy LM, Davis M, Krystal JH, et al (1995b): Yohimbine facilitated acoustic startle in combat veterans with post-traumatic stress disorder. *Psychopharmacology (Berl)* 117:466–471.

Morgan CA III, Grillon C, Lubin H, Southwick SM (1997): Startle reflex abnormalities in women with sexual assault-related posttraumatic stress disorder. *Am J Psychiatry* 154: 1076–1080.

Ornitz EM, Pynoos RS (1989): Startle modulation in children with posttraumatic stress disorder. *Am J Psychiatry* 146:866–870.

Orr SP, Pitman RK, Lasko NB, Herz LR (1993): Psychophysiological assessment of posttraumatic stress disorder imagery in World War II and Korean combat veterans. *J Abnorm Psychol* 102:152–159.

Orr SP, Lasko NB, Shalev AY, Pitman RK (1995): Physiological responses to loud tones in Vietnam veterans with posttraumatic stress disorder. *J Abnorm Psychol* 104:75–82.

Orr SP, Solomon Z, Peri T, Pitman RK, Shalev AY (1997): Physiologic responses to loud tones in Israeli veterans of the 1973 Yom Kippur war. *Biol Psychiatry* 41:319–326.

Pallmeyer TP, Blanchard EB, Kolb LC (1986): The psychophysiology of combat induced PTSD in Vietnam veterans. *Behav Res Ther* 24:645–652.

Phillips RG, LeDoux JE (1992): Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 106:274–285.

Servatius RJ, Ottenweller JE, Bergen MT, Soldan S, Natelson BH (1994): Persistent stress-induced sensitization of adrenocortical and startle responses. *Physiol Behav* 56:945–954.

Shalev AY, Orr SP, Peri T, Schreiber S, Pitman RK (1992): Physiologic response to loud tones in Israeli patients with posttraumatic stress disorder. *Arch Gen Psychiatry* 49:870–875.

Spielberger CD (1983): *Manual for the State-Trait Anxiety Inventory.* Palo Alto, CA: Consulting Psychologist Press.

Swerdlow NR, Geyer MA, Vale WW, Koob GF (1986): Corticoreleasing factor potentiates acoustic startle response in rats: Blockade by chlordiazepoide. *Psychopharmacology (Berl)* 88:147–152.