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In the face of fear: Anxiety sensitizes defensive responses to fearful faces

Christian Grillon and Danielle R. Charney

Section on the Neurobiology of Fear and Anxiety, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Fearful faces readily activate the amygdala. Yet, whether fearful faces evoke fear is unclear. Startle studies show no potentiation of startle by fearful faces, suggesting that such stimuli do not activate defense mechanisms. However, the response to biologically relevant stimuli may be sensitized by anxiety. The present study tested the hypothesis that startle would not be potentiated by fearful faces in a safe context, but that startle would be larger during fearful faces compared to neutral faces in a threat-of-shock context. Subjects viewed fearful and neutral faces in alternating periods of safety and threat of shock. Acoustic startle stimuli were presented in the presence and absence of the faces. Startle was transiently potentiated by fearful faces compared to neutral faces in the threat periods. This suggests that although fearful faces do not prompt behavioral mobilization in an innocuous context, they can do so in an anxiogenic one.

Descriptors

Fear; Anxiety; Startle reflex; Fearful face

Fearful faces are used extensively in emotion research, probably because they have been historically conceptualized as threatening stimuli (Darwin, 1872; Ekman & Friesen, 1976) with preferential access to the fear network. This hypothetical system is proposed to be dedicated to rapid perceptual analysis of threat stimuli and activation of defensive mechanisms (fear; note that the terms *fear* and *defensive mechanisms* are used interchangeably in this article to denote mobilization of bodily resources in response to threat; Ohman, 2000). Although evidence shows that fearful faces prompt prioritized perceptual processing (Mogg, Garner, & Bradley, 2007; Stein, Zwickel, Ritter, Kitzmantel, & Schneider, 2009) and facilitates sensorimotor coupling (Ernst, Cornwell, Mueller, & Grillon, 2010; West, Al-Aidroos, Susskind, & Pratt, 2011), whether such stimuli also evoke fear remains to be determined.

For many investigators, fearful faces provide insight into perceptual processing of threat stimuli (Mogg et al., 2007; Stein et al., 2009), but for others, especially in a neuroimaging context, fearful faces entail not only prioritized perception, but also an emotional experience, fear (Hariri & Holmes, 2006; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002; Lau et al., 2009; Pine et al., 2005). This latter view is partly based on the observation

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Address correspondence to: Christian Grillon, Ph.D., NIMH/MAP, 15K North Drive, Bldg. 15K, Room 203, MSC 2670, Bethesda, MD 20892-2670. Christian.grillon@nih.gov.

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that fearful faces preferentially activate the amygdala, a structure that plays a key role in the expression of fear (Felmingham et al., 2010; Hariri & Holmes, 2006; Hariri et al., 2002). Accordingly, amygdala activation is equated with the emotional experience of fear (Felmingham et al., 2010; Hariri et al., 2002; Zink, Stein, Kempf, Hakimi, & Meyer-Lindenberg, 2010). Given the widespread use of fearful faces in emotion research (Vytal & Hamann, 2010), it is critical to understand whether such stimuli engage a defensive response characteristic of fear.

The interpretation that fearful faces elicit fear derives from the claim that biologically relevant threat stimuli prompt behavioral action (Darwin, 1872; Ohman, 2000) and that they convey strong emotional information associated with “robust visceral responses” (Hariri et al., 2002). However, this latter assertion has been questioned; it is uncertain whether pictures of fearful faces are sufficiently threatening to evoke an emotional response. Merely looking at fearful faces does not evoke autonomic response (Dunsmoor, Mitroff, & LaBar, 2009) or subjective fear (Davis & Whalen, 2001). Rather, fearful faces are important signals for potential threat in one’s environment, leading to increased vigilance for the source of danger without concomitant defensive mobilization (Whalen, 1998; Whalen et al., 1998).

Evidence that fearful faces do not evoke defensive responses comes from startle studies. The startle reflex, a reflexive reaction to an abrupt and intense stimulus, is reliably increased or potentiated by negative emotional states (Davis, Walker, Miles, & Grillon, 2010; Lang, Bradley, & Cuthbert, 1990). Startle potentiation is considered an index of fear, reflecting amygdala-mediated activation of subcortical defensive mechanisms (Davis, Walker, et al., 2010; Lang et al., 1990). The basic startle potentiation effect has been widely replicated using various procedures (Davis, Walker, et al., 2010). Importantly, startle is potentiated in the presence of a wide variety of unpleasant stimuli, including pictures depicting attacks, mutilation, and violence (Lang et al., 1990). Pictures of emotional faces have rarely been used in startle studies (Anokhin & Golosheykin, 2010; Dunning, Auriemmo, Castille, & Hajcak, 2010; Hess, Sabourin, & Kleck, 2007; Springer, Rosas, McGetrick, & Bowers, 2007). Results show that fearful faces do not potentiate startle (Anokhin & Golosheykin, 2010; Springer et al., 2007) and angry faces are only weakly associated with affective startle potentiation (Dunning et al., 2010; Hess et al., 2007; Springer et al., 2007). This differential effect of angry and fearful facial expressions could be due to the fact that angry faces signal a direct threat whereas fearful faces depict a potential threat in the environment (Whalen, 1998; Davis & Whalen, 2001), the former potentially requiring an immediate action and the latter leading to increased processing of environmental cues.

Although fearful faces may promote privileged perceptual processing without concomitant defensive responses in an innocuous context, this may not necessarily be the case when subjects are anxious. Anxiety is a state of exaggerated hypervigilance (Rosen & Schulkin, 1998) that could prioritize defensive mobilization to certain types of stimuli. For example, fear-relevant stimuli are believed to have hard-wired potential to evoke fear provided a preexisting state of elevated anxiety (Gray, 1987; Lovibond, Siddle, & Bond, 1993). Indeed, stress and anxiety potentiate more autonomic responses to fear-relevant (e.g., spider) stimuli than to fear-irrelevant stimuli (e.g., mushroom; Davey, 1992; Kartsounis, Pickersgill, & Pickersgill, 1981; Öhman, Eriksson, Fredriksson, Hugdahl, & Olofsson, 1974). Functionally, selective activation of fear mechanisms to threat stimuli would minimize false alarms and reduce metabolic demand. Accordingly, anxiety would be expected to selectively promote defensive mobilization to fearful faces as opposed to neutral faces. An alternative view is that anxiety does not prioritize response to specific threat, but rather enhances sensitivity to stimuli indiscriminately. From an evolutionary perspective, heightened sensitivity at the expense of specificity may be preferable; failure to respond appropriately could reduce chances for survival. The findings of early facilitation of sensory processing by anxiety

(Baas, Milstein, Donlevy, & Grillon, 2006; Cornwell, Echiverri, Covington, & Grillon, 2008; Cornwell et al., 2007) and reduced specificity of stimuli that evoke fear in certain anxiety disorders (e.g., posttraumatic stress disorders) are consistent with this view. Further, a recent study shows that stress increases the sensitivity at the expense of the specificity of amygdala response to emotional faces (van Marle, Hermans, Qin, & Fernández, 2009). In the present study, we used the startle reflex during experimentally induced anxiety to test these alternative hypotheses and to explore the relationship between anxiety and defense mobilization to fearful faces.

Accordingly, we combined a well-validated threat-of-shock procedure (Grillon, Ameli, Woods, Merikangas, & Davis, 1991) with a picture-viewing paradigm during which subjects were presented with fearful and neutral faces in alternating periods of safety or shock threat while receiving occasional startle stimuli. On the basis of previous studies (Anokhin & Golosheykin, 2010; Springer et al., 2007), we expected that startle reactivity between the fearful and the neutral faces would not differ in the safe condition. However, we hypothesized that startle would be increased in the threat condition compared to the safe condition and that this startle potentiation would be greater for fearful faces compared to neutral faces. To control for the potential effect of emotional arousal (as opposed to valence), happy faces were also compared to neutral faces in a within-subject design during a separate testing session. Startle was not expected to be differentially modulated by neutral and happy faces in the safe or threat condition.

Method

Participants

Participants were paid, healthy volunteers who gave written informed consent approved by the NIMH Human Investigation Review Board. Inclusion criteria included (a) no past or current psychiatric disorders as per the Structured Clinical Interview for DSM-IV (First, Spitzer, Williams, & Gibbon, 1995) given by an experienced clinician, (b) no history of mania in any first degree relatives as by self-report, (c) no medical condition that interfered with the objectives of the study as established by a physician, and (d) no use of illicit drugs or psychoactive medications as per urine screen. Trait anxiety was measured during screening using the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger, 1983). The STAI is a 20-item measure of trait anxiety with high internal consistency, good test-retest reliability and strong convergent and discriminant validity (Spielberger, 1983). Thirty-three subjects participated in the study, but 3 did not return for the second session. Two subjects were excluded for low startle reactivity (see below). The final group consisted of 28 subjects (13 women) with a mean age of 26.5 years ($SD = 4.9$ years). Subjects consisted of 2 African Americans, 7 Asian Americans, and 19 Caucasians.

Overview

The experiment consisted of two experimental sessions that examined startle reactivity during the presentation of neutral, fearful, and happy faces either when subjects were safe or at risk for shock. There were two separate sessions on different days. During one session, participants were shown neutral and fearful faces (neutral/fearful session) and during the other session they were shown neutral and happy faces (neutral/happy session).

Stimuli, Materials and Response Measurement

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments, London, UK). 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. The

raw electromyographic (EMG) signal was amplified, filtered (30–500 Hz), and digitized at 1000 Hz. The electric shock was produced by a constant current stimulator and administered on the left wrist.

The schematic of the experiment and example of stimuli are shown in Figure 1. Each visual stimulus was bordered on each vertical side by a green or blue band. The center of the stimuli (between the colored bands) consisted of either a gray square or a face, resulting in four types of stimuli: (a) a gray square bordered by green bands, (b) a face bordered by green bands, (c) a gray square bordered by blue bands, and (d) a face bordered by blue bands. Each band of a given color was associated either with the word “safe” or the word “shock?” written vertically. For half the subjects, the word “safe” was written in the green bands and the word “shock” was written in the blue bands. This order was reversed for the remaining subjects. Two categories of facial expressions from the Pictures of Facial Affect (Ekman & Friesen, 1976) were selected. One category consisted of neutral and fearful faces from six different individuals (three men). The other category consisted of neutral and happy faces expressions from six other individuals (three men). A two-button custom-built box was used for subjects’ responses.

Questionnaires

Subjects retrospectively rated their anxiety level during the safe and threat conditions on an analog scale ranging from 1 (*not at all anxious*) to 9 (*extremely anxious*). In addition, the faces were rated on dimensions of arousal and valence using the self-assessment manikin (SAM; Bradley & Lang, 1994). The level of shock pain experienced during testing was rated on an analog scale ranging from 1 (*not at all painful*) to 10 (*extremely painful*).

Procedure

Each subject participated in a screening session that included a physical examination, a psychological interview, and responses to various questionnaires prior to enrollment in the study. The order of the neutral/fearful faces session and neutral/happy faces session was counterbalanced across subjects. The time between the first and second session was 13.7 days and 12.9 days for subjects who had the neutral/fearful faces session first and the neutral/happy faces first, respectively. A given session started with a startle habituation procedure (to reduce initial startle reactivity) and a shock workup procedure. Subjects were then provided detailed information about the threat-of-shock procedures, after which the experiment began (Phase 1). Immediately following the experiment, subjects were asked to retrospectively rate their anxiety levels during the safe and threat conditions. After a 10 min break period the experiment was repeated (Phase 2) and was followed by a second retrospective rating of anxiety and a rating of the pain caused by the shock. Finally, subjects were shown each of the pictures and were asked to rate them on dimensions of valence and arousal using the SAM (Bradley & Lang, 1994).

During the startle habituation procedure, nine startle stimuli were delivered every 17–20 s (results not presented here). The shock workup procedure was initiated to set up the shock intensity at a level judged moderately painful by the subjects. Each experimental phase consisted of four habituation startle stimuli (every 17–20 s) followed by six alternating safe and threat blocks (three safe blocks and three threat blocks) each lasting approximately 170 s. For half the subjects, Phase 1 started with a safe block and Phase 2 with a threat block, and for the other half of the subjects, the reverse order was used. The same faces were used in Phases 1 and 2, but in a different order of presentation. During each block a visual stimulus signaling a safe or threat condition was continuously displayed on the monitor, and three neutral faces and three fearful faces (or happy) were presented sequentially for 6 s each with an interval of 15 to 30 s between faces (Figure 1). Seven startle stimuli were delivered

in each block, three in the absence of the faces (intertrial interval [ITI] startle) and the remaining four administered 3.5 or 4.5 s after the onset of a face, two during a neutral face and two during a fear (or happy) face. Note that startle stimuli were not administered during one fear (or happy) face and one neutral face to reduce the predictability of startle delivery. The study was designed so that the time interval between startle stimuli varied from 18 to 25 s. In each phase, a total of four shocks were administered (at least one shock per threat block). Shocks were not administered during a face or within less than 10 s following a face. No startle stimuli followed a shock by less than 10 s. To summarize, in each phase, each face was shown three times for a total of 18 neutral faces (3 in each of three safe blocks and 3 in each of three threat blocks) and 18 fearful (or happy) faces (3 in each of three safe blocks and 3 in each of three threat blocks). A startle stimulus was delivered on two thirds of these faces and 18 startle stimuli (3 per block) were delivered during the ITI.

Subjects were informed that neutral and fear or happy faces would be presented on a monitor. To ensure that they paid attention to the faces they were asked to press a button to indicate a neutral face and another button to indicate a fearful or happy face. The instructions emphasized accuracy and deemphasized speed. Subjects responded with high accuracy to the faces (correct responses for the neutral/fearful sessions: neutral faces/safe: 94.2%, neutral faces/threat: 92.9%, fearful faces/safe: 93.1%, fearful faces/threat: 93.9%, correct responses for the neutral/happy sessions: neutral faces/safe: 94.1%, neutral faces/threat: 92.4%, happy faces/safe: 93.1%, happy faces/threat: 92.1%), their response not being significantly influenced by phase, faces, or the safe/threat conditions. Subjects were told that they would receive shocks during threat conditions but not during safe conditions, and that these conditions would be signaled by the words “shock?” or “safe” written on colored vertical bands on the side of the monitor. In addition, they read written instructions that also included images of the safe and threat stimuli. It was specified that they could receive between one and three shocks in each threat block and that the shocks were administered randomly.

Data Analysis

Peak magnitude of the startle/blink reflex was determined in the 20–100-ms time frame following stimulus onset relative to a 50-ms prestimulus baseline. The raw eyeblink signal was rectified in a 150-ms window starting 50 ms before the startle stimulus and then integrated using a custom-written scoring program that simulates an integrator circuit with a 10-ms time constant. Subjects with low startle reactivity were excluded. Low startle reactivity was defined as no startle response during at least four trials during the initial startle habituation procedure. The magnitude scores were transformed into *T* scores by pooling data within session type for each subject. The data were then averaged within each condition and stimulus types (ITI, fear/happy faces, neutral faces). Data were entered into repeated measure analyses of variance (ANOVAs). Because our a priori hypothesis was that startle would be potentiated by fearful faces—but not the happy faces—compared to neutral faces in the threat but not safe condition, two separate analyses were conducted, one contrasting the neutral and fearful faces and the other contrasting neutral and happy faces using Stimulus Type (ITI, neutral, fearful, or happy) $\times$ Condition (safe, threat) $\times$ Phase (first, second) ANOVAs. Alpha was set at .05 for all statistical tests. Because a preliminary investigation revealed that the main results were not affected by order of presentation of the sessions (i.e., neutral/fearful faces on Day 1 or Day 2) or sex (except for a significant Stimulus Type $\times$ Sex effect for the neutral/fearful face session; $F[2,52] = 5, p < .02$), these two factors were not included in the ANOVAs. Greenhouse–Geisser corrections (GG- ϵ) were used for main effects and interactions involving factors with more than two levels. A measure of effect size (partial eta-squared) is included for each ANOVA.

Results

Startle Magnitude

There were significant main effects of condition, $F(1,27) = 56.8, p < .0009, \eta^2 = .68$, which reflected the expected potentiation of startle in the threat compared to the safe condition, and phase $F(1,27) = 98.0, p < .0009, \eta^2 = .78$, due to reduction of startle from Phase 1 to Phase 2 (most likely reflecting habituation). More importantly, there was a Stimulus Type $\times$ Condition $\times$ Phase, $F(2,54) = 4.0, p < .04, \epsilon = .12$, interaction. This interaction was due to differential startle potentiation to the faces during the threat condition compared to the safe condition in Phase 1 but not in Phase 2. As shown in Figure 2, in the safe condition of Phase 1, startle was not significantly modulated by the faces (ITI vs. fearful, ITI vs. neutral, neutral vs. fearful, all $p > .3$). In the threat condition of Phase 1, however, startle to the fearful face was significantly greater compared to ITI startle, $F(1,27) = 6.3, p < .02, \epsilon = .18$, and startle during the neutral faces, $F(1,27) = 18.0, p < .0009, \epsilon = .40$. Startle during ITI and during the neutral faces did not significantly differ, $F(1,27) = 0.69, n.s., \epsilon = .03$. There was a significant condition effect in Phase 2, $F(1,27) = 31.2, p < .0009, \epsilon = .54$, but no significant difference among startle evoked during ITI, neutral faces, and fearful faces in the safe or threat conditions of Phase 2 (all $p > .5$).

During the neutral and happy faces session there was no startle modulation by the happy faces (Figure 3). There were significant main effects of condition, $F(1,27) = 13.7, p < .0009, \eta^2 = .34$, due to greater overall startle in the threat compared to the safe condition, block, $F(1,27) = 41.1, p < .0009, \eta^2 = .60$, due to reduction of startle from Phase 1 to Phase 2 (habituation), and a significant Condition $\times$ Block interaction, $F(1,27) = 7.3, p < .02, \eta^2 = .21$. This latter effect reflected an overall reduced degree of startle potentiation in Phase 2, condition main effect, $F(1,27) = 5.7, p < .02, \eta^2 = .18$, compared to Phase 1, condition main effect, $F(1,27) = 16.5, p < .0009, \eta^2 = .38$. The Stimulus Type $\times$ Condition and Stimulus Type $\times$ Condition $\times$ Phase interactions were not significant, $F(2,54) = 0.7, n.s., \eta^2 = .01$ and $F(2,54) = 0.7, n.s., \eta^2 = .01$, respectively.

Ratings

The anxiety ratings are shown in Table 1. The subjective anxiety ratings for each session were entered into separate Condition (safe, threat) $\times$ Phase (first, second) ANOVAs. As expected, subjects reported more anxiety during the threat condition compared to the safe condition. This was reflected in the neutral/fearful faces and the neutral/happy sessions by a significant main effect of condition, $F(1,27) = 104.2, p < .0009, \eta^2 = .79$, $F(1,27) = 118.2, p < .0009, \eta^2 = .81$, respectively. Phase and the Condition $\times$ Phase interaction were not significant (all $p > .2$).

As expected, fearful faces were less pleasant ($M = 3.3, SD = 0.8$ vs. $M = 5.0, SD = 0.6$) and more arousing ($M = 3.8, SD = 2.1$ vs. $M = 1.8, SD = 0.9$) than the neutral faces, $t(27) = 9.9, p < .0009$, and $t(27) = 6.3, p < .0009$, respectively, and the happy faces were more pleasant ($M = 6.5, SD = 0.9$ vs. $M = 4.4, SD = 0.7$) and more arousing ($M = 3.8, SD = 1.9$ vs. $M = 2.6, SD = 1.3$) than the neutral faces, $t(27) = 11.5, p < .0009$, and $t(27) = 3.7, p < .001$, respectively. The arousal ratings were not significantly different for fearful ($M = 3.8, SD = 2.8$) and happy ($M = 3.8, SD = 1.9$) faces, $t(27) = .01, n.s.$

Correlations

We found that startle was selectively increased by fearful faces (relative to the neutral faces) in Phase 1 of the threat condition. Startle potentiation scores, reflecting the increase in startle from the neutral to the fearful face in the threat condition (Phase 1), were calculated (startle during fearful faces minus startle during neutral faces). There was a significant (Pearson)

positive correlation between the startle potentiation score and the increased fear reported by the subjects during the threat condition relative to the safe condition ($r = .45$, $p < .02$; Figure 4), suggesting that increasing levels of anxiety during the threat blocks were associated with increasing levels of startle potentiation to the fearful faces.

Discussion

The main finding of this study was that fearful faces potentiated startle only in the threat condition of Phase 1. Fearful faces did not potentiate startle in the safe condition of Phase 1 or in the safe and threat conditions of Phase 2, suggesting that the priming of defensive response was short lived and context dependent. Startle was not modulated by happy faces in any of the conditions. The finding that startle was not modulated by happy or fearful faces in the safe condition is consistent with prior observations in innocuous contexts; it confirms that fearful faces do not inherently evoke a defensive response (Anokhin & Golosheykin, 2010; Springer et al., 2007) but can do so in a threatening context. The differential startle response to the neutral and fearful faces in the threat condition is inconsistent with the hypothesis that anxiety sensitizes defensive mobilization indiscriminately. Rather, it supports the hypothesis that anxiety selectively activates defensive mechanisms to potentially harmful stimuli.

These results suggest that threatening stimuli such as fearful faces, which reliably activates the amygdala in neuroimaging studies, do not necessarily evoke fear. In fact, the magnitude of amygdala activation seems to be a poor predictor of defensive activation. Fearful faces evoke greater amygdala activation than unpleasant pictures from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 1994; see Hariri et al., 2002). Yet, only unpleasant IAPS pictures reliably potentiate startle (Lang, Bradley, & Cuthbert, 1990). It is more likely that amygdala activation to fearful faces reflects the extraction of emotional information and the prioritization of sensory processing based on the affective significance of the stimulus (Pessoa & Adolphs, 2010; Vuilleumier, Armony, Driver, & Dolan, 2001). Under innocuous circumstances, privileged amygdala processing of mild threat can lead to facilitation of nondefensive motor action (e.g., saccade; Ernst et al., 2010; West et al., 2011), but not facilitation of defensive responses.

There are several possible explanations for the anxiety-induced selective potentiation of startle to fearful faces compared to neutral faces. The affective modulation of startle by negative pictures is dependent on a certain arousal level evoked by the pictures (Cuthbert, Bradley, & Lang, 1996); startle is potentiated by highly arousing but not lowly arousing negative pictures. Given the key role of arousal in startle potentiation, it is possible that negative stimuli that are intrinsically not sufficiently arousing to potentiate startle could do so to the extent that a certain level of arousal of the organism is reached (e.g., following administration of drugs that modulate arousal). Accordingly, one could speculate that, in the present study, arousal inherent to fearful faces was too low to potentiate startle in the safe condition, but that the anticipation of shocks raised the subjects' arousal to a level sufficient to prompt startle potentiation. This view assumes that the affective modulation of startle by a picture depends on the additive effects of arousal evoked by the picture and the level of arousal of the subjects. However, this potential additivity effect remains to be demonstrated; so far, drugs that reduce or increase subjects' arousal have not been shown to affect the affective modulation of startle by pictures (Buchanan, Brechtel, Sollers, & Lovallo, 2001; Schachinger et al., 2008). In other words, the arousal prerequisite for startle potentiation may refer to the subjective arousal evoked by the picture (which is likely to be correlated with the unpleasantness of the picture), not to subjects' arousal state (Schachinger et al., 2008). These observations suggest that increase in arousal per se from the safe to the threat conditions was not likely to be responsible for the present findings.

Alternatively, at a psychological level, fearful faces operate as biologically relevant (e.g., threatening) signals in the environment (Davis & Whalen, 2001). Such signals have been associated with covariation bias, defined as the tendency to overestimate the association between threat stimuli and negative outcomes (see Tomarken, Mineka, & Cook, 1989). In the threat condition, the fearful faces may have functioned as signals for a proximal threat; subjects may have believed that they were at increased risk of shock in the presence of a fearful face, prompting increased defensive responding. Covariation bias is a characteristic of individuals with an anxiety disorder or high in trait anxiety (e.g., de Jong & Merckelbach, 1993). Whether a threat of shock can promote covariation bias in nonanxious subjects remains to be demonstrated, but this is a real possibility. We recently reported that, in healthy volunteers, sustained shock anticipation mimics some of the cognitive symptoms of anxiety disorders (Robinson, Letkiewicz, Overstreet, Ernst, & Grillon, 2011).

Another possibility follows the proposal that emotional faces operate as extinguished conditioned stimuli (CS; Kim et al., 2004; Kim, Somerville, Johnstone, Alexander, & Whalen, 2003). A key characteristic of extinction is that it suppresses conditioned fear responses, but it does not erase the memory of the CS–danger association. As a result, extinction is context dependent (i.e., a CS predicts harm in the acquisition context but not in the extinction context). Following extinction, conditioned fear can return under certain circumstances, especially in stressful contexts (reinstatement, spontaneous recovery; Bouton, 2002). In the present study, the stress of shock anticipation may have prompted reinstatement of an extinguished fear response to fearful faces. Interestingly, the bed nucleus of the stria terminalis (BNST), a structure involved in startle potentiation (see below), plays a key role in reinstatement (Waddell, Morris, & Bouton, 2006). Hence, shock anticipation may have induced a BNST-dependent anxiety state that facilitated prepotent defensive responses to threat cues such as fearful faces.

Startle was potentiated by the fearful faces in the threat condition in Phase 1 but not in Phase 2. This transient effect could also be interpreted in the context of fearful-faces-as-extinguished-CS hypothesis. Because reinstated CS are fragile and extinguished rapidly (Bouton, 2002), the reinstatement may not have survived the repeated presentations of the fearful faces (which predicted no aversive outcome) in Phase 2. It is also possible that if subjects initially expected a shock to follow fearful faces (covariation bias), they may have understood rapidly that these faces were not predictive of the shocks (leading to a loss of startle potentiation). Another possibility, given that startle potentiation is dependent on the amygdala, is that amygdala reactivity to the fearful faces was also transient. Indeed, several neuroimaging studies have pointed out that amygdala responses to salient stimuli, including emotional faces, habituate rapidly (Breiter et al., 1996; Phelps et al., 2001). The lack of startle potentiation to the fearful faces in Phase 2 may indicate that these stimuli no longer activated the amygdala.

At a neural level, rodent studies have long demonstrated a key role for the extended amygdala (amygdalae nuclei and BNST) in emotional processing and defensive mechanisms. More specifically, the basolateral complex (BLA) coordinates a network of cortical areas during stimulus evaluation (Pessoa & Adolphs, 2010). The BLA is also the primary input site of the amygdalae, whereas the central nucleus of the amygdala (CeA) and the BNST are the main output sites, sending efferent signals to downstream structures (including brain stem and hypothalamic and basal forebrain targets) responsible for the expression of fear (Davis, Walker, et al., 2010; Davis & Whalen, 2001). Thus, the BLA receives sensory information whereas the CeA and BNST mediate defensive responses to threat (Davis, Walker, et al., 2010). With this model as a framework for interpreting the present results, we can speculate that when the organism feels safe, the BLA preferentially encodes the threat value of mildly threatening stimuli such as fearful faces. For example,

faces appraised negatively, including fearful faces, activate the ventral amygdala, a structure within which the BLA is located (Kim et al., 2003, 2004; Whalen et al., 2001). However, the strength of fearful face-mediated BLA activation may be too weak to reach the threshold of activation of the CeA/BNST and concomitant defense mechanisms. Threat of shock, however, primes CeA/BNST-mediated defensive mechanisms, which then may become more sensitive to weak BLA input to which they previously did not react, including mildly aversive stimuli such as fearful faces.

An alternative explanation is that under threat of shock the CeA and BNST remain insensitive to weak sensory input, but that shock anticipation boosts perceptual processing of the fearful faces, increasing perceptual input to a level sufficient to activate the CeA or BNST. The difference between these two possibilities is that the former hypothesizes that threat of shock sensitizes only neural structures involved in defensive responses (i.e., CeA/BNST) whereas the latter assumes that the threat of shock sensitizes both perceptual processing and defensive responses mechanisms. There is evidence that threat of shock facilitates perceptual processing (Cornwell et al., 2007, 2008). Threat of shock activates a neural system encompassing the amygdala, insula, and dorsal ACC (Mechias, Etkin, & Kalisch, 2010). This neural system could facilitate visual processing, enhancing the processing of fearful faces via reentrant afferent from the amygdala at different levels of the visual stream (Vuilleumier, 2005), including early visual processing regions (e.g., V1, V4; Damaraju, Huang, Barrett, & Pessoa, 2009) and areas specialized in face processing (e.g., fusiform face area; Vuilleumier et al., 2001; Vytal & Hamann, 2010). Ultimately, as spatial resolution improves (Davis, Johnstone, Mazzulla, Oler, & Whalen, 2010; Etkin et al., 2004; Gamer, Zurowski, & Buchel, 2010), neuroimaging studies will shed light on the brain network underlying the present results.

Despite strong evidence of the role of the amygdala in the expression of fear, including potentiated startle, in rodents, it should be noted that threat-of-shock procedures lead only to weak amygdala activation in functional magnetic resonance imaging (fMRI) studies (Mechias et al., 2010) and that lesions of the amygdala following fear learning do not prevent the expression of fear-potentiated startle in nonhuman primates (Antoniadis, Winslow, Davis, & Amaral, 2007, 2009; but see Funayama, Grillon, Davis, & Phelps's 2001 study that shows that lesions of the temporal lobe suppress potentiated startle in humans). It is thus possible that structures other than the amygdala are responsible for startle potentiation. Potential mediators of potentiated startle include the BNST and the orbitofrontal cortex (Antoniadis et al., 2009; Kalin, Shelton, & Davidson, 2007).

The results of the current study should be considered within the context of its strengths and limitations. The study had several strengths. First, we used a within-subject design, which reduces variability and increases the power to detect significant effects. Second, the potential effect of arousal was controlled by testing happy faces in addition to fearful faces. Finally, anxiety was induced with a robust and well-validated paradigm that reliably increases subjective anxiety and potentiates startle. Among the limitations is the fact that we did not get separate ratings of arousal and valence for each type of facial expression in the safe and threat conditions. The ratings were obtained prior to the experiments and may not reflect subjects' feelings during the experiment, especially in the threat condition. Given (a) that the fearful faces potentiated startle in the threat but not safe condition (Phase 1) and (b) the key role of arousal and valence in mediating startle potentiation (Cuthbert et al., 1996), it would have been informative to examine whether rating of arousal and valence were affected by shock anticipation. A second limitation is the relatively lack of power to detect small effects. However, the absence of affective modulation of startle to the fearful and happy faces in the safe condition does not seem to be due to a lack of power. Rather, it is consistent with published reports (see the Introduction). A final limitation is the use of a retrospective

questionnaire to assess anxiety during the safe and threat conditions. Retrospective questionnaires are affected by factors such as the absence of clear anchors and the time elapsed since the event being rated. However, in the present study the time between the end of the test and the ratings was kept at a minimum, and the study used a within-subject design, which minimizes issues associated with anchors.

A clear implication of our finding is that stress and anxiety do not indiscriminately sensitize defensive responses to external stimuli; anxiety prioritizes defensive mobilization to potential threat. One can speculate that, in nonanxious subjects in an innocuous context, fearful faces lead to increased stimulus processing in an attempt to elucidate the sources of threat, whereas in a threatening context, fearful faces prompt a more immediate behavioral need for action (fight or flight). It is possible that even when safe anxious individuals (e.g., patients with an anxiety disorder) react with both heightened attentional bias together with motivational need for defensive responding. This is suggested by findings that adults and children with anxiety disorders show enhanced amygdala activation to fearful face at baseline (Felmingham et al., 2010; Shin et al., 2005; Thomas et al., 2001). As indicated above, greater amygdala activation does not necessarily imply greater fear response. However, given the present findings and the fact that anxiety disorders can be conceptualized as a state of exaggerated anxiety, it is possible that this greater amygdala activation reflects both attentional bias and increased behavioral fear expression. Future studies should examine whether startle is potentiated by fearful faces in innocuous contexts in patients with an anxiety disorder in order to test the hypothesis that such faces evoke qualitatively different reaction in healthy individuals and anxious patients.

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Figure 1.

Schematic of one phase of the experiment. There were two testing sessions, one using neutral and fearful faces (shown here) and one using neutral and happy faces. Each session was comprised of two successive safe/threat phases separated by a 10-min rest period. Each phase started with a safe (shown here) or a threat block. There were three safe blocks and three threat blocks per phase, each block lasting 170 s. During each block, three neutral faces and three fearful faces were shown. Each face was shown for 6 s.

Figure 2. Startle magnitude during ITI, neutral faces, and fearful faces in the first (Safe 1/Threat 1) and the second (Safe 2/Threat 2) phases of the neutral/fearful faces session. *Significant ($p < .0009$) difference in startle magnitude during fearful face compared to ITI and neutral faces.

Figure 3. Startle magnitude during ITI, neutral faces, and happy faces in the first (Safe 1/Threat 1) and the second (Safe 2/Threat 2) phases of the neutral/happy faces session.

Figure 4. Startle potentiation during the fearful faces (relative to the neutral faces) plotted against subjective anxiety during the threat condition (relative to safe condition) in Phase 1.

Table 1

Mean (SD) Trait Anxiety, Subjective Anxiety, and Shock Pain Ratings

Sessions	Trait anxiety	Subjective anxiety				Shock pain
		Safe 1	Threat 1	Safe 2	Threat 2	
Neutral/fearful	31.3	1.8 (1.1)	5.3 (2.0)	1.5 (1.0)	5.0 (2.0)	6.8 (1.3)
Neutral/happy	(6.4)	1.8 (1.1)	5.3 (1.9)	1.6 (0.9)	5.2 (1.8)	6.6 (1.2)