COGNITIVE REGULATION AND REVERSAL OF FEAR: REFLEX PHYSIOLOGY AND NEURAL ACTIVATION

By

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Fear and its amelioration are central to understanding and treating a number of emotional disorders, including phobias and anxiety. This underscores the clinical relevance of understanding how the brain mitigates fear. It also raises questions about the neural basis of fear and about what brain circuits confer the ability to adjust fear behaviors flexibly when circumstances change. The two experiments described in this dissertation (1) determined if verbal instructions are effective in both eliciting and terminating defensive physiological reactions and (2) elucidated the neuroanatomy of instructed fear, by comparing neural activity when participants were threatened with electric shock and viewed unpleasant pictures. Results indicate language can flexibly control defensive reactions, by altering neural activation in brain structures and circuits that are commonly engaged in different aversive contexts.

CHAPTER 1
INTRODUCTION

Fear is a construct used to describe a constellation of behavioral, physiological, and evaluative responses that occur when an organism encounters an aversive stimulus. Aversive cues are theorized to activate a defensive neural system that is supported by phylogenetically primitive neural circuits (Lang & Bradley, 2010; Bradley, 2009; Lang & Davis, 2006; Lang, Bradley & Cuthbert, 1997; Dickinson & Dearing; Konorski, 1967). Activation of motivational circuits is presumed to feedback to sensory and motor systems that heighten information processing and mediate responses that have evolved to escape harm (Lang & Bradley, 2010). In animals, activation of the defensive system ultimately mediates fight or flight responses, including behavioral "freezing" (e.g., hiding) and counter-threat displays. In humans, many overt responses associated with fearful percepts, thoughts, and images are typically delayed, modulated, or altogether inhibited. Instead, what persists and forms the basis of emotional experience and expression are physiological changes and reflexive behaviors that index preparation for action. From this perspective, fear in humans can be described as an action disposition (Fridja, 1986).

Classical fear conditioning has proven to be one of the most effective experimental tools for studying fear and anxiety, and has enabled the integration of animal and neuroimaging research to identify overlap in the representative physiology and behavior of fear. It involves pairing the presentation of an initially neutral stimulus with the presentation of an unconditioned aversive stimulus (US), such as an electric shock. Through repeated pairings, the presentation of the conditioned stimulus (CS) comes to elicit anticipatory responses indicative of fear and defensive activation. In simple

conditioning, a single CS is repeatedly presented and conditioning effects are indexed by subtracting baseline levels of a measure from level changes elicited by the CS. In discriminative conditioning paradigms, there are generally two conditioned stimuli, one paired with the US (CS+) and one unpaired (CS-), and learning is assessed by contrasting responses to the CS+ and CS-.

Although the paired presentation of a CS+ and US generally prompts fear learning, direct experience does not always play a role in human fear acquisition. In fact, research into the etiology of specific fears has noted that many fears are learned vicariously, through observation or symbolic learning (Rachman, 2002; Antony & Barlow, 2002). In addition, while classical fear conditioning has proven to be a highly effective paradigm in studying emotional learning in animals, fear conditioning in humans is often difficult because truly aversive reinforcers cannot be administered ethically. Because of this, many participants simply fail to learn or remain unaware of the aversive contingencies.

This has led researchers to develop the instructed fear paradigm, in which verbal instructions inform participants that a certain stimulus is associated with the possibility of an aversive event (e.g., electric shock). In this paradigm, participants are instructed that following the presentation of a particular visual cue there is the potential to receive a painful electric shock, while another cue is specified as a safety signal. The term “instructed fear conditioning” was coined to note that instructions rather than exposure to an aversive US were used to establish fear associations, but also to emphasize the assumed conceptual and neural overlap with mechanisms of classical fear conditioning

(Grillon, Ameli, Merikangas, Woods & Davis, 1993; Grillon, Ameli, Woods, Merikangas & Davis, 1991).

Previous studies have repeatedly found that verbal threat, which maximizes expectancy and awareness of the learning contingencies, is sufficient to initiate and sustain fear related changes in behavior and physiology. Cook and Harris (1937) first reported verbal conditioning of electrodermal reactions and subsequent studies have similarly found that explicit knowledge about the threat contingencies enhances conditioning of autonomic activity (Olsson & Phelps, 2004; Lipp & Edwards, 2002; Soares & Ohman, 1993; Dawson & Schell, 1985; Hughdal, 1978; Hughdal & Ohman, 1977; Grings & Dawson, 1973)—especially in comparison to fear responses acquired through classical conditioning (Hughdal & Ohman, 1977; Lipp & Edwards, 2002).

More recently Grillon and colleagues (1993, 1991) demonstrated that threat of shock also reliably potentiates the reflexive eye blink response to an acoustic startle probe, compared to probes presented during safe periods. Additional studies indicated that in addition to startle potentiation, threat of shock also heightens skin conductance change and corrugator muscle activity (i.e., frowning), and prompts increased cardiac deceleration (e.g., Bradley, Silakowski & Lang, 2008; Bradley, Moulder, & Lang, 2005). Together these studies encourage a parallel between instructed and classical fear conditioning, since they both elicit similar somatic and autonomic defensive reactions. The finding that threat of shock potentiates startle blink magnitude seems especially supportive, since startle potentiation is widely used to index defensive activation in both animals (for a reviews see Davis, 2006; Davis, Antoniadis, Amaral & Winslow, 2008) and humans (Grillon & Baas, 2003; Lang, 1995; Lang, Bradley & Cuthbert, 1990).

Fear and its amelioration are central to understanding and treating a number of emotional disorders, including phobias and anxiety. A natural extension of the demonstrated effectiveness of instructions in eliciting fear is to determine whether instructions are equipotent in attenuating defensive reactions. Much of what is known about the mechanisms of extinction learning has been revealed using classical learning procedures. In this case, fear extinction involves repeated presentations of the fear-eliciting cue in the absence of aversive reinforcement. In threat of shock paradigms, however, such a scenario would simply reflect a continuation of the experiment, because an aversive, unconditioned stimulus is rarely (if ever) presented. One way to study extinction following threat of shock is to provide explicit information that the threat and safety contingencies have been reversed, so that a previously threatening cue no longer signals the possibility of receiving an electric shock. Experiment 1 uses a reversal procedure to examine whether instructions can be used to elicit and terminate a broad array of defensive reactions, including startle blink magnitude, skin conductance change, corrugator muscle activity, and heart rate.

Consistent with the interpretation that these physiological changes index defensive activation, a similar profile of physiological changes occurs when participants view unpleasant pictures. For instance, it has been demonstrated repeatedly that the startle blink reflex is potentiated when participants view unpleasant, compared to neutral or pleasant, pictures (Bradley, Codispoti & Lang, 2006; Bradley, Codispoti, Cuthbert & Lang, 2001; Lang, 1995; Lang et al., 1990; Vrana, Spence & Lang, 1988). Perception of emotionally arousing, unpleasant media also prompts increased cardiac deceleration, heightened corrugator muscle activity (e.g., frowning), and increased skin conductance

(Bradley et al., 2001; Lang, Greenwald, Bradley & Hamm, 1998). The overlap in the defensive psychophysiology for threat of shock and aversive perception suggests these two processes may engage similar neural circuits and structures related to activation of the defensive motivational system. A direct comparison of the brain regions activated in both induction contexts would foster a better understanding of which brain regions likely mediate the aversiveness of threat of shock, rather than anticipatory or learning related processes.

Spurred by psychophysiological validation of the instructed fear paradigm, numerous studies have examined the neural bases of instructed fear. The results of these studies, using either positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), largely overlap with neuroimaging findings for classical fear conditioning. Threat of shock is consistently linked to increased activation of the anterior insula, anterior cingulate, superior medial frontal gyrus (i.e., dorsomedial prefrontal cortex), inferior frontal gyrus (i.e., dorsolateral and ventrolateral prefrontal cortex), and lateral orbitofrontal cortex (e.g., Drabant, Kuo, Ramel, Blechert, Edge, Cooper, Goldin, Hariri & Gross, 2011; Butler, Pan, Tuescher, Engelien, Goldstein, Epstein, Weisholtz, Root, Protopopescu, Cunningham-Bussel, Chang, Xie, Chen, Phelps, Ledoux & Silberweig, 2007; Kumari, Ffytche, Das, Wilson, Goswami & Sharma, 2007; Dalton, Kalin, Grist, & Davidson, 2005; Phelps, O'Connor, Gatenby, Gore, Grillon & Davis, 2001). One exception is that, unlike classical fear conditioning, threat of shock does not reliably lead to increased amygdala activation (Mechias, Etkin & Kalisch, 2010; cf., Phelps et al., 2001). Since clinical and neuroscientific research has cast the amygdala in a pivotal (and often exclusive role) in mediating fear learning, it has been difficult to

discern which other brain regions activated during threat of shock specifically relate to defensive activation.

Experiment 2 attempts to resolve this issue by directly comparing brain structures and circuits activated under threat of shock with those activated during aversive perception. The principal aims of Experiment 2 are to determine (a) if these different aversive events engage the same or different brain structures and circuits, and (b) if viewing unpleasant pictures under threat of shock has a cumulative effect on the neural activity of structures commonly activated during aversive perception and threat of shock.

Because threat of shock involves a nonspecific description of a painful US, the imagined aversiveness of the US will likely vary across individuals as a function of their fear of pain. Consistent with this idea, participants reporting high dental fear exhibited increased startle potentiation and autonomic reactions when threatened with electric shock (Bradley et al., 2008) and also show heightened defensive reactions during narrative imagery of scenarios involving invasive dental procedures (McNeil, Vrana, Melamed, Cuthbert & Lang, 1993). Experiment 2 built on these findings and assessed functional brain activity during threat of shock in participants reporting high and low dental fear. To the extent that activity in specific regions reflects affective distress, high fear individuals are expected to show enhanced neural activation in regions activated both during threat of shock and aversive perception.

CHAPTER 2
KNOWING WHAT TO FEAR AND WHEN TO FEAR IT: DEFENSIVE REFLEXES ACURATELY TRACK REVERSAL OF THREAT CONTINGENCIES

Fear learning allows an organism to use environmental cues to predict upcoming aversive events. As discussed in Chapter 1, much of what is known about fear learning derives from the use of classical fear conditioning protocols, in which neutral stimuli are repeatedly paired with the presentation of an unconditioned, aversive stimulus (e.g., electric shock). Yet many human fears are acquired through observation, verbal communication, or symbolic learning rather than direct experience of an aversive event (Rachman, 1977, 2002), and, as already discussed in Chapter 1, simply being instructed that a cue signals the possibility of shock is sufficient to heighten defensive reflexes and activate defensive neural circuits.

Can instructions similarly attenuate defensive reactions if circumstances change and threatening cues are now safe? One way to study extinction following threat of shock is to provide explicit information that a cue no longer signals the possibility of receiving an electric shock. While this manipulation may seem trivial, the use of instructions to lessen anticipatory fear underlies many cognitive-behavioral therapies and cognitive emotion regulation strategies.

Prior studies have found that instructions are effective in attenuating a variety of fear responses, both when fear is acquired through classical conditioning or instructions. Most studies have demonstrated a marked reduction in electrodermal activity when participants are explicitly informed that a cue will no longer be paired with shock (Olsson & Phelps, 2004; Lipp & Edwards, 2002; Soares & Ohman, 1993; Dawson & Schell, 1985; Hughdal, 1978; Hughdal & Ohman, 1977; Grings, 1973; Grings & Dawson, 1973; Cook & Harris, 1937). Other studies have used instructions to extinguish

fear conditioned heart rate responses (Chatterjee & Eriksen, 1962; Notterman, Schoenfeld & Bersh, 1952), conditioned suppression of button pressing (Bond, 1979; Di Giusto & Bond, 1978), and conditioned eyelid responses using air puffs (Nicholls & Kimble, 1964; Norris & Grant, 1948).

An interesting variation on the use of instructions to terminate autonomic fear responses was first reported by Wilson (1968) and replicated by Grings and colleagues (1973). Following the acquisition of conditioned electrodermal activity through discriminative conditioning, participants received instructions that reversed the significance of the conditioned stimuli. Specifically, they were informed that shock would now only occur following the stimulus previously unpaired with shock. In actuality, however, no additional shocks were delivered. This paradigm allowed simultaneous assessment of fear excitation and inhibition. Results indicated that, immediately following reversal of the shock contingencies, electrodermal responses decreased for the cue previously paired with shock and increased for the cue that had previously signaled safety. Using the same paradigm, an immediate reversal of skin conductance activity to cues that previously signaled threat or safety was also found when the conditioned stimuli were pictures of snakes and spiders (McNally, 1981).

Experiment 1 used a novel adaption of the instructed reversal paradigm described by Wilson (1968). In this design, participants were initially told either that the two different colors of the presented cues (e.g., red or blue) signaled the possibility of receiving an electric shock or safety, or that the two different shapes of the presented cues (e.g., square, circle) signaled threat or safety. Halfway through the experiment, new instructions were given that the other perceptual feature (e.g., color or shape) now

indicated whether a cue signaled threat of shock or safety. As a result of these instructions, half of the cues reversed their prior associations with threat and safety, while the remaining half retained a prior association with threat or safety.

By using multiple perceptual features to define threat and safe cues, physiological changes could be compared for threat and safe cues that reversed in meaning and directly compared—within participants—to reactivity when cues consistently signaled threat or safety. Also, in comparison with prior studies that only examined skin conductance activity, defensive reactions were more broadly assessed in Experiment 1 to better characterize the pattern of autonomic and somatic physiological changes that accompany instructed fear reversal. A full array of measures that are known to index emotional engagement were assessed: skin conductance, heart rate, corrugator electromyographic (EMG) activity and the reflexive blink response to an acoustic startle probe.

Prior studies have demonstrated that different physiological measures reflect different aspects of defensive engagement (e.g., Low, Lang, Smith & Bradley, 2008). For instance, elevated skin conductance activity reflects increased activity of the sympathetically innervated eccrine sweat glands (Dawson, Schell & Filion, 2007) and is related to response mobilization in both appetitive and aversive contexts (Bradley et al. 2001; Lang, Bradley & Cuthbert, 1997). On the other hand, the cardiac deceleration typically seen under threat of shock mainly reflects parasympathetic activity (Berntson, Boysen, Bauer & Torello, 1989; Campbell, Wood & McBride, 1997) and indexes increased attention to threatening stimuli in the form of heightened sensory intake and orienting (Bradley, Moulder & Lang, 2008; Bradley, 2009). Because previous studies

found that instructed extinction or reversal of shock contingencies decreased autonomic reactions, it was expected that changes in skin conductance and heart rate would reflect the current threat contingencies.

Unlike autonomic indices such as heart rate and skin conductance, changes in facial expression are under greater voluntary control—consistent with their role in social communication—and generally vary with the hedonic valence of the eliciting context. For instance, the corrugator supercillii muscles are responsible for contraction of the eyebrows and when the motor response is large enough, produce an identifiable frown. Corrugator activity is therefore useful as an index of defensive activation and increases during perception of unpleasant pictures (Bradley et al., 2001; Lang et al., 1998) or when threatened with electric shock (Bradley et al., 2006; Shackman, Maxwell, McMenamin, Greischar & Davidson, 2011). If a reversal of the threat contingencies results in lingering defensive activation, it is expected that corrugator activity may remain heightened for safe cues that were previously threatening. If instead, corrugator EMG activity primarily reflects the current contingencies, differences between threat and safe cues are expected regardless of whether their original meaning is reversed or maintained.

Previous research has repeatedly found that the eye blink component of the human startle response—a defensive reflex—is potentiated in an aversive stimulus context (Bradley et al., 2006; Bradley et al., 2001; Lang, 1995; Bradley, Lang, & Cuthbert, 1993; Lang et al., 1990). Affective startle modulation is interpreted as an instance of motivational priming and regarded as an index of defensive activation when probed in the context of aversive cue (Lang et al., 1997, 1990). The blink response to

an acoustic startle probe differs from other physiological responses in that is fast and automatic (Bradley et al., 2006; Anthony, 1985). Thus, of particular interest in the current study is whether potentiation of the startle blink response will be completely attenuated when probed during cues that previously signaled threat of shock, or whether the initial association with threat will result in lingering potentiation of the startle reflex.

There is some evidence to support the view that the startle reflex will remain potentiated when cues that initially signaled threat of shock become safety signals. Counterconditioning and discrimination reversal learning studies indicate that initial aversive conditioning interferes with performance of appetitive learning and behaviors (Bouton, 1993; Peck & Bouton, 1993), and is interpreted as evidence that appetitive and defensive motivational systems reciprocally inhibit one another (Dickerson & Dearing, 1979; Konorski, 1967). Prior studies in humans have also found that extinction training attenuates but never completely abolishes fear-potentiated startle (Norrholm, Jovnovic, Vervliet, Myers, Davis, Rothbaum & Duncan, 2006; Norrholm, Vervliet, Jovanovic, Boshoven, Myers, Davis, Rothbaum & Duncan, 2008; Kindt, Soeter & Vervliet, 2008). Together these findings suggest that an initial association with threat may interfere with the ability of the same cue to later signal safety.

However, in several of the studies discussed above, instructed extinction or reversal of conditioned electrodermal reactions is reported to occur within one or two trials into the extinction or reversal phases (Lipp & Edwards, 2002; Soares & Ohman, 1993; McNally, 1981; Hughdal, 1978; Hughdal & Ohman, 1977; Grings et al., 1973; Wilson, 1968). Instructions that no additional shocks would occur are also found to

hasten extinction of conditioned skin conductance responses in comparison to when extinction is learned through repeated, unreinforced presentations of a conditioned stimulus (e.g., Olsson & Phelps, 2004; Lipp & Edwards, 2002; Soares & Ohman, 1993; Hughdal & Ohman, 1977; Grings & Dawson, 1973). Moreover, previous studies in animals (Myers & Davis, 2004; Winslow, Noble & Davis, 2008) and humans (Jovanovic, Keyes, Fiallos, Myers, Davis & Duncan, 2005; Jovanovic, Norrholm, Keyes, Fiallos, Meyers, Davis & Duncan, 2006) have demonstrated sizeable attenuation of fear-potentiated startle the first time a safety signal and danger cue are presented together in a conditional discrimination procedure (Jovanovic et al., 2005; Jovanovic et al., 2006; Meyers & Davis, 2004). Interestingly, in humans, successful conditioned inhibition of the startle reflex is related to knowledge about which stimulus predicts aversive reinforcement or safety (Jovanovic et al., 2006). This suggests that when participants are explicitly told that a threat cue no longer signals danger and instead signals safety, blink magnitude may no longer show signs of potentiation and will be equivalent in magnitude with blinks elicited during cues that always signaled safety.

Method

Participants

Seventy-one students (46 female and 25 male; *M* age = 19.6, *SD* = 2.04) participated in partial fulfillment of a requirement for the introductory psychology course at the University of Florida. Informed consent was obtained as stipulated by the University of Florida Institutional Review Board.

Due to equipment or experimenter error, data for two male participants were excluded entirely, and data for one female participant was excluded from the analysis of heart rate. The final 69 participants were included in all analyses.

Materials and Design

Stimuli

Stimuli were geometric shapes (cubes, spheres) colored either red or blue. Color (red/blue) and shape (cube/sphere) were covaried to form 4 sets of distinct perceptual cues that included 128 unique, abstract objects. The paradigm is illustrated in Figure 2-1. In the initial learning phase, differences in one feature—either color or shape—cued threat of shock or safety. For example, one group of participants were told that any time a red stimulus appeared, an electric shock was possible, whereas if it was blue, there was no possibility of receiving an electric shock.

In the reversal phase (halfway through the experiment), a second set of instructions shifted attention to the other perceptual dimension to identify whether a cue signaled threat of shock or safety. To continue with the present example, participants who were initially instructed that red and blue cued threat and safety, were given a second set of instructions that the shape of the object cued threat or safety—for example a cube indicated the possibility of an electric shock, while a sphere indicated safety. As a result of these instructions, half of the cues (e.g., red cubes and blue spheres) retained their previous association with threat or safety, while the remaining half reversed their affective associations. This resulted in four sets of cues: those which always signaled threat of shock (e.g., red cubes), those which always signaled safety (e.g., blue spheres), safe cues that reversed in meaning to signal threat of shock (e.g., blue cubes), and threat cues that that reversed in meaning to signal safety (red spheres). The specific colors and shapes associated with threat and safety in each phase were counterbalanced across participants.

A total of 48 trials were split up into two phases—instantiation and reversal. Each phase was subdivided into six blocks each containing 4 trials that included one of each cue type. The serial position of each cue presentation was pseudo-randomized to limit consecutive presentations of a particular shape and color. Each cue was presented for 12 s followed by an interstimulus interval (ITI) varying between 12 and 18 s in length. PC-compatible computers running Presentation (Neurobehavioral Systems, Inc., Albany, CA) and VPM software (Cook, 2001) were used to control stimulus presentation and collect all physiological measures. All visual cues and text instructions were projected onto a canvas screen measuring 121 cm x 182 cm using an LCD projector.

Startle probes were 96 db, 50 ms bursts of white noise generated by a Coulbourn S81-02 noise generator and gated by a Coulbourn S82-24 (Coulbourn Instruments, Whitehall, PA) audio-mixer amplifier. Startle probes were delivered through Telephonics TDH-96 (Farmingdale, NY) earphones. Startle probes were presented on 32 of the 48 trials at 4.5, 5.5, 6.5, or 7.5 s following cue onset.

A single very mild electric shock (20 ms duration, 1.6 mA) was delivered at the end of the experiment to avoid deception. The shock was generated by an ML408 isolated physiological stimulator (ADInstruments, Inc., Colorado Springs, CO) under TTL control and connected to a bar electrode that was attached to the posterior surface of the right wrist.

Questionnaires

Following completion of the experiment participants rated the unpleasantness of shock anticipation and shock exposure. Participants also reported the feature associations described in each set of instructions, whether or not they believed they

were going to be shocked during the experiment (87% of participants believed they would be shocked), and completed a basic demographic questionnaire.

Procedure

After the sensors were attached, the experimenter instructed participants about the initial set of shock contingencies. These instructions emphasized that whenever a stimulus appeared on-screen and contained a specific perceptual feature, it was possible that an electric shock would be delivered through the electrode attached to their wrist. Participants were given no additional information about the shock (e.g., when it would occur or its intensity). The experimenter repeated these instructions twice to clarify the shock contingencies and asked follow-up questions that verified participants' understanding of each cue's initial meaning. The experimenter then attached the stimulating bar electrode to the participant's right wrist. Participants were further instructed to view each stimulus in its entirety and to maintain their gaze on a fixation cross presented at the center of the monitor.

Immediately prior to the beginning of the experiment, instructions that described the initial shock contingencies were displayed for 15 s on the screen. Midway through the experiment, a second set of instructions appeared on the screen for 15 s and informed participants that a different perceptual feature now indicated whether a cue signaled threat of shock or safety.

Data Acquisition

Startle blink magnitude

The startle reflex was recorded using two small Ag/Ag-Cl electrodes placed over the left orbicularis oculi muscle. Raw orbicularis activity was acquired at 8-1000 Hz using a Coulbourn S75-01 bioamplifier and sampled at 2000 Hz from 100 ms prior to

probe onset to 250 ms after probe onset. Offline the digitized signal was filtered from 28-500 Hz (Blumenthal, Cuthbert, Fillion, Hackley, Lipp & von Boxtel, 2005) using a Hamming windowed, non-recursive bandpass filter, and then rectified and smoothed using a Butterworth filter with a 20 ms time constant.

Skin conductance

Skin conductance was measured using two large Ag/Ag-Cl electrodes filled with 0.05 NaCl paste (TD-246; Mansfield R & D, St. Albans, VT), placed adjacently over the hypothenar eminence of the left palm. A constant current (.5 V) was generated between the electrodes using a Coulborn S71-22 coupler. Activity was sampled and digitized at 20 Hz and offline, averaged into half-second bins.

Corrugator electromyography

Activity over the corrugator supercilli muscle above the left eye was measured with small Ag/Ag-CL electrodes. The raw electromyographic signal was bandpass filtered from 90-1000 Hz using a Coulbourn S75-01 bioamplifier, rectified and integrated using a Coulbourn S76-01 contour following integrator with a 500 ms time constant, sampled at 20 Hz, and offline, averaged into half-second bins.

Heart rate

The electrocardiogram was recorded from the left and right forearms, using large Ag/Ag-CL electrodes and a Coulbourn S75-01 bioamplifier with a bandpass filter of 8-40 Hz. Raw electrocardiogram activity was sampled at 500 Hz. Offline R-wave spikes were registered using an algorithm implemented in MATLAB that mimics a Schmitt trigger. Interbeat intervals were calculated, converted into heart rate in beats per minute (bpm; Graham (1978), and averaged into half second-bins.

Data Reduction

Blink magnitude was scored offline using a peak scoring algorithm described by Globisch and colleagues (1993) implemented in MATLAB. Trials with an onset of less than 20 ms or excessive baseline activity were omitted from the analysis (less than 2% of trials). To assess reactions during the threat and safe periods, each half-second bin of corrugator, skin conductance, and heart rate activity was deviated from a 1 s baseline prior to cue onset and averaged across the 12 s trial.

Data Analysis

For each physiological measure, two mixed-model analyses of variance were separately computed for each phase (instantiation and reversal) and included repeated measures of Cue (threat, safe), and Contingency (maintain, reverse), as well as Gender (male, female) as a between participants factor. In the reversal phase, cues were coded as either threat or safe based on the current set of contingencies. Coded in this manner, a main effect of Cue in the reversal phase indicated a significant difference in reactivity during threat and safety, regardless of whether the cue contingencies from the instantiation phase were maintained or reversed. On the other hand, an interaction of Contingency and Cue indicated that reactions to threat and safe cues differed based on whether a cue had maintained or reversed its prior association with threat or safety

Results

Table 2-1 lists mean skin conductance change, corrugator change, heart rate change, and startle eye blink responses during threat and safe periods as a function of Phase (instantiation, reversal), Contingency (maintain, reverse), and Cue (safe, threat). There were no main effects or interactions involving gender for any measure other than corrugator activity.

Blink Reflex Magnitude

Figure 2-2 illustrates the magnitude of the blink reflex to startle probes presented during threat and safe periods. In the instantiation phase, blink magnitude was larger during cues that signaled threat of shock, compared to those that signaled safe periods ($F(1,67) = 49.73$, $p < .0001$). As expected, startle magnitude during threat and safety in the instantiation phase did not differ based on whether meaning of a cue would be maintained or reversed in the second phase (Cue x Contingency, $F = 1.1$, *ns*).

In the reversal phase, overall, blink magnitude was larger during threat relative to safe cues (Cue, $F(1,67) = 21.65$, $p < .0001$) and there was no interaction of Cue and Contingency ($F < 1$, *ns*). This indicated that startle magnitude was potentiated when a cue signaled threat regardless of whether it maintained or changed its original meaning, and that a prior association with threat or safety did not interfere with startle modulation as a function of the current contingencies. Not surprisingly, when cues retained their original meaning, blink reflexes remained potentiated during threat compared to safe cues ($t(68) = 3.53$, $p < .001$). Startle was also potentiated for threat cues that had previously signaled safety, and was attenuated for safe cues that previously signaled threat of shock ($t = 3.49$, $p < .001$). Moreover, startle attenuation during safe cues that had initially been threatening was equivalent to blinks elicited during cues that always signaled safety. Likewise, startle magnitude was equally potentiated during threat cues that had initially signaled safety and those that had always signaled shock.

To assess how quickly the startle reflex decreased in magnitude when a cue no longer signaled threat of shock, blink magnitude was compared for individual startle probe presentations in the reversal phase. From the very first probe presented, blink magnitude during cues that had previously signaled threat of shock was attenuated

compared to cues that always signaled threat of shock (all $t < -2.47$, ps $< .05$). These tests indicated an early and sustained attenuation of startle. Similarly, beginning with the first startle probe presentation, safe cues, that had previously signaled threat, elicited blinks that were equivalent in magnitude with those elicited during cues that had always signal safety (all $t < 1$, ps $> .32$).

Corrugator Muscle Activity

As illustrated in Figure 2-3 A, corrugator muscle activity in the instantiation phase was heightened under threat of shock compared to safety (Cue, $F(1,67) = 5.83$, $p < .05$). As expected, corrugator activity during threat and safety did not differ based on whether cue contingencies would eventually be reversed or maintained (Cue x Contingency, $F < 1$, *ns*).

In the reversal phase, corrugator tension was again heightened under threat of shock compared to safe periods (Cue, $F(1,67) = 5.85$, $p < .05$). Again this effect did not differ as a function of whether the contingencies were reversed or maintained. (Contingency x Cue, $F < 1$, *ns*). When the meaning of a cue was unchanged, threat cues heightened corrugator tension relative to safe cues ($t(68) = 2.02$, $p < .05$). Moreover, threat cues that had previously signaled safety elicited increased frowning, compared to a decrease in corrugator tension during safe cues that initially signaled threat ($t = 2.75$, $p < .01$). This decrease in corrugator tension did not differ from that elicited by cues that consistently signaled safety ($t < 1$, $p = .51$). Similarly, threat cues elicited equivalent increases in corrugator activity whether they initially signaled safety or had always signaled threat of shock ($t < 1$, $p = .69$).

Replicating previous findings (Bradley, Codispoti, Sabatinelli & Lang, 2001), women were more facially expressive than men, with greater overall corrugator activity

both in the instantiation ($F(1,67) = 10.22$, $p < .005$) and reversal phase ($F = 4.24$, $p < .05$). Although the interaction of Gender and Cue did not reach significance ($F(1,67) = 1.75$, $p = .19$) pairwise comparisons suggested that women specifically showed greater corrugator activity under threat of shock than men ($t(67) = 3.0$, $p < .005$).

Skin Conductance

In the instantiation phase, skin conductance was larger in the context of threat cues compared to safe cues ($F(1,67) = 63.27$, $p < .0001$), as illustrated in Figure 2-3 B. As expected, there were no differences in skin conductance activity as a function of whether a cue would reverse or maintain its meaning in the reversal phase (Cue x Contingency, $F < 1$, *ns*).

In the reversal phase, overall, threat cues elicited greater sympathetic activation relative to safe cues (Cue, $F(1,67) = 8.6$, $p < .005$). The lack of an interaction of Contingency and Cue ($F < 1$, *ns*) indicated that threat-related changes in skin conductance did not depend on whether the cue contingencies were reversed or maintained across phases. When cue meanings were held constant, threat cues elicited greater sympathetic activity than safe cues ($t(68) = 2.63$, $p < .05$). When the cue contingencies were reversed, skin conductance activity increased during threat cues that had previously signaled safety, and decreased during safe cues that had initially signaled threat ($t = 2.76$, $p < .05$). Safe cues, that initially signaled threat or that had always signaled safety elicited equivalent changes in skin conductance ($t < 1$, $p = .39$). Similarly, the observed increase in sympathetic activation during threat cues was equivalent between cues that initially signaled safety and those that always signaled threat of shock ($t < 1$, $p = .45$).

To determine if there was an immediate reduction in skin conductance when a cue no longer signaled threat of shock, skin conductance change was compared for each block of trials in the reversal phase. Replicating previous studies (McNally, 1981; Grings et al., 1973; Wilson, 1968), in the first block of the reversal phase, cues that had previously threatened shock elicited less sympathetic activation than cues that always signaled threat ($t(68) = 2.69$, $p < .01$), and importantly, were equivalent to the changes in skin conductance elicited for cues that always signaled safety ($t < 1$, $p = .91$; Figure 2-4).

Heart Rate

In the instantiation phase, threat cues prompted heart rate deceleration that was sustained for the entire threat period, whereas safe cues prompted a small, initial acceleration that was not sustained (see Figure 2-3 C*; *$F(1,66) = 19.15$, $p < .0001$). As expected, cardiac deceleration did not differ as function of future cue contingencies (Cue x Contingency, $F = 2.18$, p = .14).

In the reversal phase, threat cues continued to prompt sustained, cardiac deceleration relative to safe cues (Cue, $F(1,66) = 11.11$, $p < .005$). Regardless of whether the contingencies had been reversed or maintained, changes in heart rate consistently indexed heightened sensory intake and orienting when threatened with shock (Cue x Contingent, $F = 1.32$, $p = .15$). When cues maintained their original meaning, threat cues prompted more cardiac deceleration than safe cues. Likewise, threat cues that had initially signaled safety also prompted greater heart rate deceleration relative to safe cues that had originally signaled threat of shock ($t(67) = -3.47$, $p < .001$). Heart rate was equivalent during safe cues that initially signaled threat or that had always signaled safety ($t < 1$, $p = .53$). Threat cues that initially signaled

safety and cues that always signaled threat prompted equivalent cardiac deceleration (*t* < 1, *p* = .41)**.**

Emotional Ratings

Participants rated their anticipation of shock (*M* = 4.06, *SD* = 2.3) as more unpleasant than actual exposure to the shock delivered at the end of the experiment (*M* = 5.4, *SD* = 1.9; *F*(1,67) = 20.6, p <.001). Shock anticipation ratings did not differ between men and women.

Discussion

Instructions are Effective In Eliciting and Terminating Defensive Reactions

Threat of shock was associated with larger increases in skin conductance, corrugator muscle activity, heart rate deceleration, and potentiation of the startle reflex compared to safe cues, suggestive of defensive mobilization. These defensive reactions were found in both the instantiation and in the reversal phase, and did not depend on whether a cue had maintained or reversed its initial association with threat or safety.

The pattern of defensive reactivity found here is generally consistent with previous studies reporting startle potentiation, increased sympathetic activation, fear bradycardia, and frowning activity during threat of shock (Bradley et al., 2005, 2008). A similar defensive profile is found during anticipation of respiratory distress (Lang, Wangelin, Bradley, Versace, Davenport, & Costa, 2011; Melzig, Michalowski, Holtz, & Hamm, 2008) and during the perception of unpleasant pictures (e.g., Bradley et al., 2001; Lang et al., 1998). Thus, this pattern of physiological change generally characterizes a state of heightened vigilance towards aversive cues in preparation for action (Lang & Bradley, 2010; Bradley, 2009; Lang et al., 1997).

Informing participants about the cue contingencies was not only effective in eliciting new fear reactions, it was also effective in terminating previous fear associations. When cues that had initially signaled threat of shock reversed in meaning and became safety signals, there was no evidence of their initial association with threat of shock. Instead there were marked decreases in skin conductance activity, frowning activity, heart rate deceleration, and blink magnitude, compared to threat cues. More importantly, responses elicited during cues that no longer signaled threat of shock were sufficiently attenuated in the reversal phase so as to be equal in magnitude with responses elicited by cues that had always signaled safety. Although past studies have reported that instructions facilitate the extinction or reversal of aversively conditioned autonomic responses (for review see Dawson & Schell, 1985), the present study demonstrates that verbal instructions are effective in broadly attenuating defensive reactions, including measures that directly index defensive engagement (e.g., startle potentiation and corrugator muscle tension). Since different physiological measures are assumed to index different aspects of defensive engagement (Low et al., 2008; Lang & Bradley, 2010), the broad attenuation of reflex physiology found here likely reflects decreased activity in multiple regions involved in processing unpleasant events.

It is known that learning conflicting hedonic associations impedes learning of contingency reversals and delays appropriate changes in conditioned responding (Bouton, 2007; Bouton 1993; Peck & Bouton, 1990)—especially when learning that a threatening cue newly predicts safety or reward. However, startle inhibition in the context of safe cues that had initially signaled threat was complete, and did not result in lingering potentiation. In fact, startle reflexes were immediately attenuated following

instructions that previously threatening cues now signaled safety. A sudden decline in startle magnitude is not entirely surprising. Extinction or reversal of conditioned skin conductance responses (Lipp & Edwards, 2002; Hughdal & Ohman, 1977; McNally, 1981; Grings et al., 1973; Wilson, 1968), lever pressing (Di Giusto & Bond, 1978), and eye blink responses (Nicholls & Kimble, 1964) are reported to occur within one or two trials following explicit instructions that terminate or alter the expectancy of aversive reinforcement.

An immediate shift in startle reactivity and skin conductance following reversal of the threat and safe contingencies, accompanied by the complete reversal of corrugator tension and heart rate change, contrasts with the slower reversal of skin conductance activity during Pavlovian fear conditioning. Schiller and colleagues (2008) found that when reinforcement contingencies were reversed it took at least six trials before skin conductance responses reflected the updated shock contingencies. Skin conductance responses and fear-potentiated startle are similarly protracted in humans during extinction learning (Schiller, Monfils, Raio, Johnson, Ledoux & Phelps, 2010; Norrholm et al., 2006; Norrholm et al.,, 2008; Kindt et al., 2008). This suggests knowing in advance that a cue no longer signals threat of shock serves to hasten fear reversal. Consistent with this idea, autonomic reactions extinguished at a faster rate when participants were instructed about the extinction contingencies, compared to when they were implicitly learned through repeated non-reinforced presentations of a conditioned stimulus (Hughdal & Ohman, 1977; Lipp & Edwards, 2001).

One reason that instructions may facilitate fear reversal is that it allows the revised contingencies to be completely learned before encountering cues that could

activate competing initial associations with threat. Receiving information regarding the current set of contingencies allowed stimulus encoding to proceed without retrieving previous associations with threat or safety. Moreover, the shift to a new feature dimension likely also increased attention to the new feature that signaled threat or safety in the reversal phase (relative to the feature that did so in the instantiation phase). Compare this with reversal of Pavlovian conditioned fear, in which the important changes in the reinforcement contingencies are not immediately signaled. In this case, a previously reinforced stimulus initially retrieves its aversive association, even though it is no longer reinforced—at least for the first few trials. Likewise, a previously unreinforced stimulus will retrieve its inhibitory association until it is experienced in an aversive context and fear learning occurs. Learning a contingency reversal in this manner will therefore depend on the rate of reinforcement, and it is well known that counterconditioning and extinction learning are slowed when initial, aversive associations are learned using partial reinforcement (Bouton, 2007).

Interestingly, most studies that have examined fear extinction or reversal in humans have taken advantage of this phenomenon and almost exclusively used partial reinforcement schedules. This suggests that using instructions to reverse stimulus associations with threat and safety might approximate learning using continuous reinforcement. For example, within one or two trials, both animals and humans quickly learn to shift behavioral responses when previously rewarding choices are followed by punishment in serial reversal learning tasks (Ghahremani, Moterosso, Jentsch, Bilder & Poldrack, 2010).

A second explanation for the rapid and complete reversal of defensive reactions seen here is that the reversal phase represented a change in context, which may have facilitated responding based on the current threat and safety contingencies. In animals changes in context are known to enhance learning that a fear conditioned stimulus signals safety (Bouton, 1993), reward (Peck & Bouton, 1990), or a change in the magnitude of unconditioned stimulus (e.g., stronger or weaker shock; Swartzentruber & Bouton, 1986). Interestingly, context changes during counterconditioning facilitate the suppression of defensive behaviors (e.g., freezing), while appetitive responses are acquired (e.g., head jerk; Peck & Bouton, 1990).

Bouton (2004; 1993) has theorized that a change in the meaning of a conditioned stimulus encourages encoding of the context, to bolster learning about the current reinforcement contingencies. The passage of time itself indirectly produces a change in the background context, based on ongoing internal and external cues. In the present study, interruption of the experiment to inform participants about the new contingencies could have signaled a change in context that facilitated learning. This is only a preliminary conclusion, as we did not directly manipulate the learning context in Experiment 1. However, the fact that prior studies which used instructions to quickly reverse fear reactions similarly involved a brief interruption of the experiment (Wilson, 1968; Grings et al., 1973; McNally, 1981), somewhat supports this view. Future studies that directly manipulate the context following contingency reversals will be useful in understanding how changes in context might enhance the effectiveness of instructions on fear learning.

Summary

When informed that a cue signaled the probability of receiving a potentially painful electric shock (that had not yet been experienced) strong autonomic and defensive reactions were elicited that indicate activation of defensive neural circuits (Lang & Bradley, 2010). Following instructions that reversed the threat and safe contingencies, there was an immediate shift in defensive reactivity suggesting that verbal instructions are effective in eliciting and terminating defensive reactions in multiple response systems. Of particular interest is the finding that for measures typically modulated by hedonic content—startle reflex and corrugator activity—an initial association with threat of shock did not result in lingering aversive responses when that same cue reversed in meaning and became a safety signal. This corroborates earlier findings that examined instructed extinction and reversal of conditioned autonomic responses, and suggests that advance knowledge about possible threats facilitates the flexible control of fear. Moreover, Experiment 1 demonstrates the instructed fear reversal paradigm can be usefully applied to better understand how fear and anxiety impact the ability to respond adaptively to salient environmental changes.

Table 2-1. Autonomic and somatic reactions during threat and safe periods before and after instructed reversal of the threat and safe contingencies.

	Instantiation				Reversal				Overall			
	Threat		Safe		Threat		Safe		Threat		Safe	
Startle blink magnitude (μV)												
Maintain	22.9	(3.0)	11.9	(1.9)	13.8	(2.2)	8.8	(1.5)	18.3	(2.5)	10.4	(1.6)
Reverse	22.7	(3.1)	13.2	(2.1)	14.3	(2.4)	9.2	(1.7)	18.5	(2.6)	11.2	(1.7)
Corrugator EMG (μV)												
Maintain	.49	(.09)	.19	(.08)	.60	(.10)	.33	(.09)	.55	(.08)	.26	(.07)
Reverse	.50	(.11)	.25	(.09)	.63	(.09)	.35	(.09)	.57	(.08)	.30	(.07)
Heart rate (bpm)												
Maintain	-1.7	(.06)	-.46	(.02)	-.82	(.02)	-.23	(.02)	-1.2	(.22)	-.35	(.25)
Reverse	-2.1	(.05)	-.15	(.02)	-1.00	(.03)	.19	(.01)	-1.6	(.24)	.02	(.20)
Skin conductance (μS)												
Maintain	.14	(.03)	-.01	(.01)	.04	(.02)	-.01	(.01)	.09	(.02)	-.01	(.01)
Reverse	.15	(.03)	-.02	(.01)	.04	(.02)	-.01	(.01)	.10	(.02)	-.01	(.01)

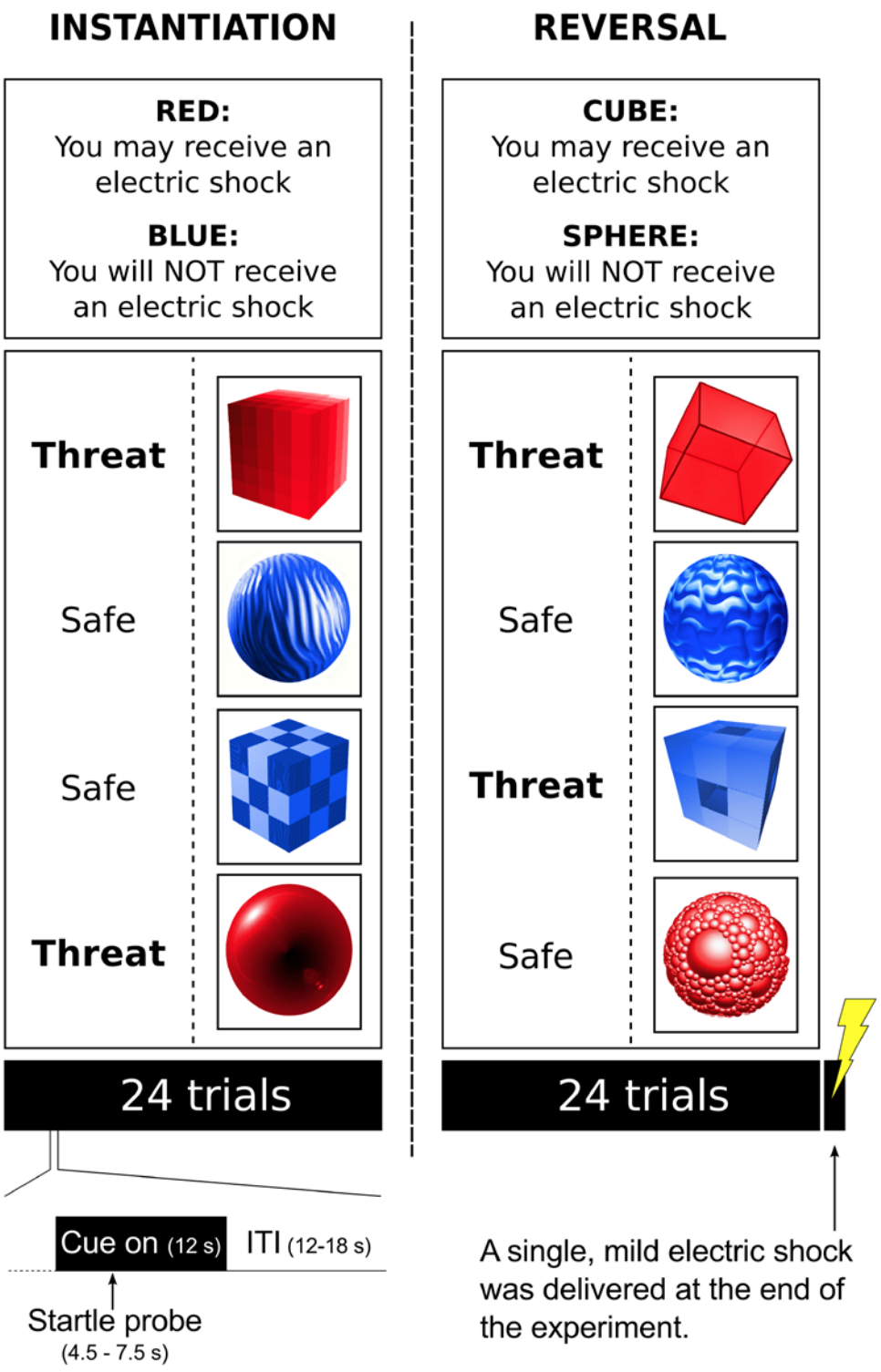

Figure 2-1. The instructed fear reversal paradigm.

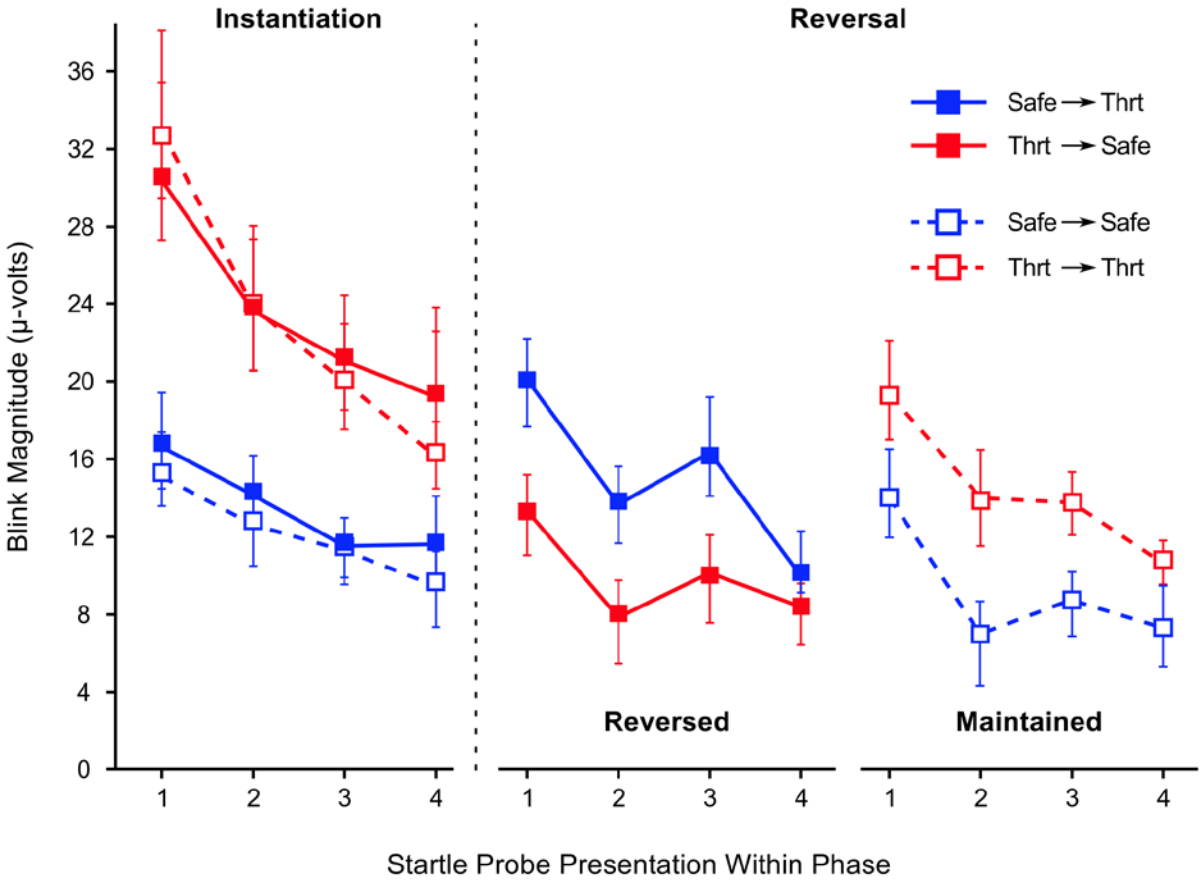

Figure 2-2. Startle blink magnitude was larger during threat, compared to safe periods and quickly decreased when cues that initially signaled threat became safety signals in the reversal phase. Blink magnitude is plotted to startle probes presented in the instantiation (left) and reversal (right) phases. In the reversal phase blink magnitude is separately plotted for cues whose initial associations with threat or safety were either reversed or maintained. Error bars represent standard error of the mean.

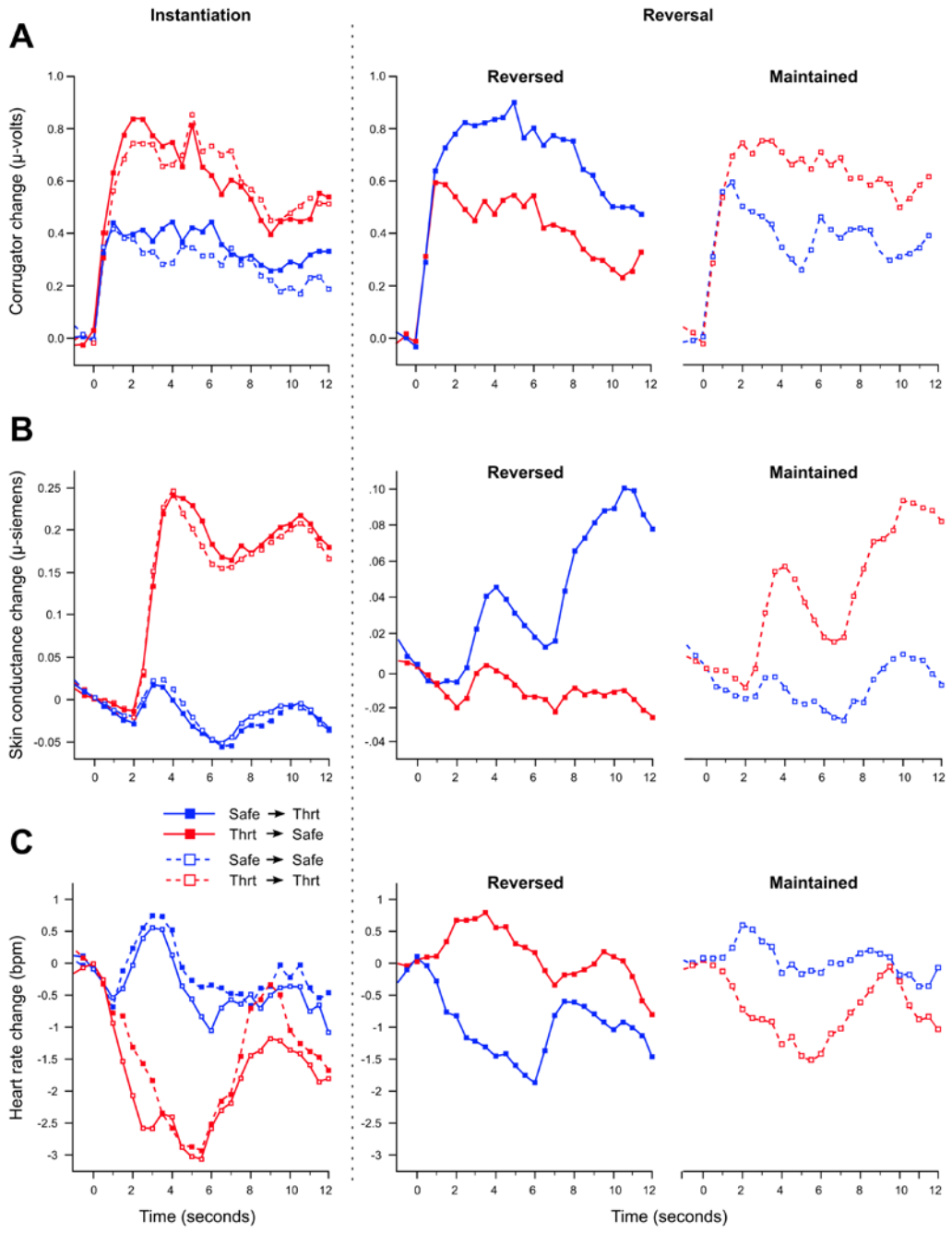

Figure 2-3. Threat of shock heightens defensive reactions both before and after instructions that reversed the threat contingencies. A) Mean change in corrugator activity increased during threat, compared to safe periods, both in the instantiation and reversal phases. B) Mean change in skin conductance level is heightened during threat, compared to safe periods, also in both phases. C) Mean heart rate showed deceleratory changes during threat, compared to safe periods, in each phase.

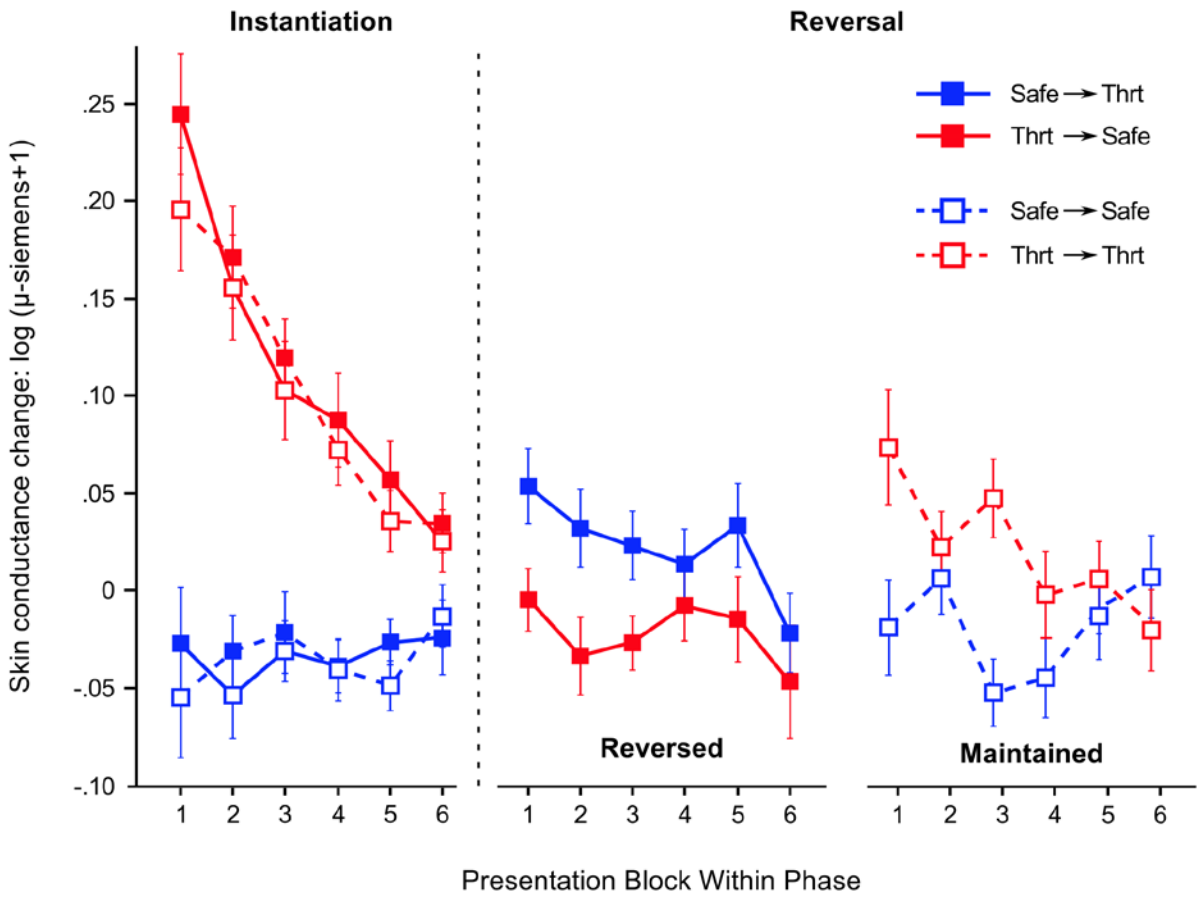

Figure 2-4. Mean skin conductance change was larger during threat, compared to safe periods and accurately tracked reversal of the threat contingencies. Mean skin conductance change is plotted for each trial block in the instantiation (left) and reversal (right) phases. In the reversal phase blink magnitude is separately plotted for cues whose initial associations with threat or safety were either reversed or maintained. Error bars represent standard error of the mean.

CHAPTER 3
AVERSIVE PERCEPTION, THREAT OF SHOCK, AND FEAR OF PAIN ACTIVATE COMMON AND DISTINCT FUNCTIONAL NETWORKS IN THE BRAIN

Defensive changes in behavior, reflex physiology, and brain activity are elicited by a number of experimental procedures that involve exposure to aversive stimuli. Two paradigms especially effective in evoking defensive reactions in the laboratory are threat of shock and the perception of emotionally arousing, unpleasant pictures (e.g., scenes of mutilated bodies or threatening situations). As discussed previously, in the threat of shock paradigm, participants are instructed that a neutral stimulus signals the possibility of receiving an aversive stimulus (e.g., receipt of a painful electric shock) and capitalizes on humans' ability to learn through verbal communication. The data from Experiment 1 indicate that threat of shock readily potentiates the reflexive eye blink component of the human startle response, heightens skin conductance and corrugator muscle activity (e.g., frowning) and prompts increased cardiac deceleration.

Similar defensive reactions are elicited when participants view unpleasant pictures. It has been repeatedly demonstrated that the startle blink reflex is potentiated when participants view unpleasant, compared to neutral or pleasant, pictures (Bradley, Codispoti & Lang, 2006; Bradley, Codispoti, Cuthbert & Lang, 2001; Lang, 1995; Lang, Bradley & Cuthbert, 1990; Vrana, Spence & Lang, 1988). Perception of emotionally arousing, unpleasant media also prompts increased cardiac deceleration, heightened corrugator muscle activity (e.g., frowning), and increased skin conductance activity (Bradley et al., 2001; Lang et al., 1998).

The similarity in physiological reactions elicited during shock anticipation and unpleasant picture processing suggests they may have a common motivational foundation. Indeed, prior neuroimaging studies and meta-analyses that have examined

neural activity during threat of shock (e.g., Drabant et al., 2011; Mechias, Etkin & Kalisch, 2010; Schunck, Erb, Mathis, Jacob, Gilles, Jacques, Namer, Meier & Luthringer, 2008; Butler, Pan, Tuescher, Engelien, Goldstein, Epstein, Weisholtz, Root, Protopopescu, Cunningham-Bussel, Chang, Xie, Chen, Phelps, Ledoux, Silbersweig, 2007; Hasler, Fromm, Alvarez, Luckenbaugh, Drevets & Grillon, 2007; Dalton, Kanlin, Grist, Davidson, 2005; Phelps et al., 2001) or emotional perception (e.g., Sabatinelli, Fortune, Li, Siddiqui, Krafft, Oliver, Beck & Jeffries, 2011; Sabatinelli, Lang, Bradley, Costa & Keil, 2009; Sabatinelli, Lang, Bradley, Costa & Versace, 2007; Sabatinelli, Bradley, Fitzsimmons & Lang, 2005; Bradley, Sabatinelli, Lang, Fitzsimmons, King & Desai, 2003; Phan, Wager, Taylor & Liberzon, 2002; Lane, Reiman, Bradley, Lang, Ahern, Davidson & Schwartz, 1997) report at least some overlap in the brain structures activated in these two tasks. Threat of shock is consistently linked to increased activation of the anterior insula, superior medial frontal gyrus (i.e., dorsomedial prefrontal cortex), and inferior frontal gyrus (i.e., dorsolateral and ventrolateral prefrontal cortex). These same regions also show heightened activation during passive viewing of unpleasant pictures. This suggests that functional activity in these regions may be similarly enhanced in different defensive contexts. This view is supported by evidence that the anterior insula, superior medial frontal gyrus, and inferior frontal gyrus are activated in a diverse number of aversive contexts (Craig, 2009; Kober, Barrett, Joseph, Bliss-Moreau, Lindquist & Wager, 2008; Phan et al., 2002).

There are also differences in the activation patterns elicited by threat cues and unpleasant pictures. Functional imaging studies of emotional perception repeatedly reported increased activation in amygdala, ventral visual cortex, and dorsal parietal

cortex when viewing emotional pictures (e.g., Sabatinelli, Lang, Bradley, Costa & Keil, 2009; Sabatinelli, Bradley, Fitzsimmons & Lang, 2005), consistent with a reentrant model of visual emotional perception (Sabatinelli et al., 2009; Spiegler & Mishkin, 1981; Amaral & Price, 1984). In contrast, increased activation in amygdala and secondary visual areas is not commonly reported during threat of shock (Mechias, Etkin & , 2010; cf. Phelps et al., 2001), and although parietal lobe activation is observed under threat of shock, it is generally confined to inferior parietal regions (e.g., supramarginal and angular gyrus). Threat of shock is reported to uniquely activate the anterior portion of the superior frontal gyrus and adjacent middle frontal gyrus. Activation of these regions during fear conditioning has been linked to expectancy and awareness of the reinforcement contingencies (Carter, O'Doherty, Seymour, Koch & Dolan, 2006; Knight, Cheng, Smith, Stein & Helmstetter, 2004).

The aim of Experiment 2 was to determine the neural circuit activated by threat of shock and to directly compare it—within participants—to patterns of brain activation during aversive perception to determine (a) if these different aversive events engage the same brain structures and circuits, (b) if processing of multiple aversive cues (e.g., unpleasant pictures under threat of shock) has a cumulative effect on blood oxygen level-dependent (BOLD) signal change, and (c) if individual differences in fear of pain modulate BOLD activity during threat of shock.

In Experiment 2, unpleasant and neutral pictures were viewed under threat of shock or safety. Two colors (red or blue) cued whether the participant might receive an electric shock, or that no shock would be delivered. Color cues were presented as a frame surrounding an unpleasant or neutral picture, or in the absence of a picture. Since

cue meaning was not systematically related to the hedonic content of the picture, we were able to identify brain regions activated during threat of shock or aversive perception using statistically independent contrasts that mirrored comparisons used in previous studies. To determine brain regions activated during threat of shock, we compared signal change during threat versus safe cues when cues were presented without a picture; which is the standard comparison used in studies investigating threat of shock. To determine regions activated during aversive perception, we compared signal change during unpleasant and neutral pictures when the colored frame surrounding a picture signaled safety; which is most similar to the standard comparison used in previous studies examining emotional perception.

Based on previous studies, we hypothesized that a network of brain regions would show increased activation both during threat periods and during aversive perception. The structures most likely to be activated in both contexts are the anterior insula, superior medial frontal gyrus, and inferior frontal gyrus, consistent with their reported activation in a variety of induction contexts. It is also clear from prior studies that threat of shock and aversive perception might activate distinct brain structures and circuits related to differences in induction contexts that target anticipation versus perception. Based on previous data, threat cues and unpleasant pictures are expected to differ in modulating BOLD signal change in the amygdala and visual areas.

If threat of shock and aversive perception activate a common functional network, another key question is whether these regions show enhanced activation when an unpleasant picture is viewed under threat of shock. In a previous study that examined defensive reflexes when unpleasant pictures cued threat of shock, no additional

increase in startle potentiation was found compared to when the same pictures signaled safety (Bradley et al., 2005). This suggests saturation in the cortical and limbic regions that mediate affective modulation of the startle reflex, as previously reported in rodents (Walker & Davis, 2002). Based on these data, one hypothesis is that regions activated both by threat cues and unpleasant pictures will not show an additional increase in activity when unpleasant pictures are viewed under threat.

Yet, in the same study (Bradley et al., 2005) other defensive reflexes (e.g., increased skin conductance, frowning activity, and cardiac deceleration) were heightened when unpleasant pictures cued threat of shock. Moreover, in two studies that directly compared startle potentiation during threat of shock and aversive perception, startle blink magnitude was larger when participants are threatened with (or exposed to) electric shock compared to when they viewed unpleasant pictures (Greenwald, Bradley, Cuthbert & Lang, 1998; Lissek, Orme, Mcdowell, Johnson, Luckenbaugh, Baas, Cornwell & Grillon, 2007). Together these results suggest that unpleasant pictures viewed under threat of shock may increasingly engage the defense system, which might be more evident when threat of shock is cued using stimulus features that are independent of picture content. Thus, it is possible that viewing unpleasant pictures under threat of shock will have an additive effect on BOLD activity in regions activated by both tasks.

In a previous study, participants reporting high dental fear exhibited increased startle potentiation and autonomic reactions when threatened with electric shock, compared to low fear participants (Bradley, Silakowski & Lang, 2006). Because individual differences in dental fear are largely mediated by a fear of pain (McNeil &

Berryman, 1989), these data suggest that participants fearful of pain are more reactive when threatened with the possibility of receiving an electric shock. In Experiment 2, we therefore assessed functional brain activity during threat of shock and aversive perception in participants reporting high and low dental fear. To the extent that neural activity reflects fearfulness when anticipating a potentially painful event, we expected high fear individuals to show enhanced activation during threat of shock. Such a finding would be consistent with prior studies that have linked increased fear of pain and pain catastrophizing to increased signal change during painful stimulation (Seminowicz & Davis, 2006; Ochsner, Ludlow, Knierim, Hanelin, Ramachandran, Glover & Mackey, 2006). Also, because the effects of fearfulness on defensive reactions during threat of shock were most pronounced in woman (Bradley, Silakowski, & Lang, 2008) and previous studies have reported gender differences in dental fear and fear of pain (Heft, Meng, Bradley & Lang, 2007), Experiment 2 focused on women who reported high or low dental fear.

In addition, although threat of shock is not typically associated with increased amygdala activation in healthy controls (Mechias et al., 2010; Drabant et al., 2010; Ploghaus et al., 1999), co-activation of amygdala and anterior insula during aversive anticipation is apparent in fear and anxiety patients (e.g., Sarinopoulos, Grupe, Mackiewicz, Herrington, Lor, Steege & Nitschke, 2010; Etkin & Wager, 2007; Lorberbaum et al. 2004). Patients with specific phobias also show increased activation in the amygdala and anterior insula when looking at pictures of a feared object (Dilger, Straube, Mentzel, Fitsek, Reichenbach, Hecht, Krieschel, Gutberlet & Miltner, 2003; Larson, Schaefer, Siegle, Jackson, Anderle & Davidson, 2006; Etkin & Wager, 2007;

Lueken, Kruschwitz, Muehlhan, Siegert, Hoyer & Wittchen, 2011; Sabatinelli, Bradley, Fitzsimmons & Lang, 2005; Straube, Mentzel, Miltner, 2007). It is possible then that high fear individuals will uniquely show amygdala activation when confronted with the possibility of receiving a potentially painful shock.

Method

Participants

Thirty-four female students (*M* age = 18.8, *SD* = 0.78) from general psychology courses at the University of Florida participated for course credit or $20. Women reporting high dental fear (n = 16) were first identified based on responses to 3 items taken from the Dental Fear Survey (DFS; Kleinknecht, Klepac, and Alexander, 1973) that were included in an online prescreening questionnaire. Those who scored above the 95% percentile were classified as potentially high dental fear, contacted by phone, and invited to participate in the study. On the day of the experiment, all 16 participants completed the entire DFS. The DFS is a sensitive, reliable and valid measure of dental fear (Schuurs and Hoogstraten, 1993) and is recommended for use in research (Newton and Buck, 2000). A cutoff score of 60—equal to the 90% percentile for a large sample of female undergraduates (*n* = 3412)—categorized participants as high or low in dental fear. Four participants scored below the cutoff score (all DFS scores < 55) and were included in the low fear group during data analysis.

Low dental fear participants (n = 18) were randomly identified from the remaining set of students, contacted by phone, invited to participate, and also completed the entire DFS at the time of the experiment. One participant from this group scored above the cutoff (DFS score = 67) and was included in the high fear group during data analysis.

All participants had normal visual acuity and reported no previous experience of claustrophobia during a phone interview. Informed consent was obtained as stipulated by the local institutional review board. Data were excluded from two low fear participants in all analyses due to excessive head motion. This resulted in a final sample of 13 high fear participants and 19 low fear participants.

Materials and Design

Stimuli consisted of 64 red and blue squares equivalent in hue and color saturation. Participants were instructed that when one cue color (counterbalanced across participants) was displayed, an electric shock could be delivered through a stimulating bar electrode attached to their ankle, and that no shock was possible during presentations of the other cue color. Cues were presented for 12 s followed by a fixed 12 s inter-trial interval consisting of a black screen. A white fixation-cross appeared in the center of the screen throughout the experiment. On 32 of the 64 trials, an unpleasant or neutral grayscale picture, framed by one of the two colors was presented; on the remaining 32 trials color cues were presented in the absence of a picture. Cues were presented in 16 blocks of 4 trials, consisting of 2 threat cues (one with and without a picture) and 2 safe cues (one with and without a picture). Cue presentations were counterbalanced across blocks so that no more than three trials of threat or safety occurred in succession.

All stimuli were backward projected onto a LCD monitor (640 x 480 pixel resolution) situated behind the participant's head, and viewed using a head-coil mounted mirror (IFIS-SA, Invivo, Orlando, FL). Stimulus presentation was controlled using a PC-compatible computer running E-Prime (Psychology Software Tools, Inc., Sharpsburg, PA).

Electric shock was delivered through a bar electrode, attached to the inner surface of the participant's right leg, immediately rostral to the medial malleolus of the tibia forming the ankle joint. Shock was generated using a Powerlab stimulator attached to a ML180 Stimulus Isolator (AD Instruments, Inc., Colorado Springs, CO). Participants received one mild electric shock (2.6 mA, 250 ms duration) halfway through the experiment, approximately 10 s following the onset of a threat cue.

Questionnaires and Self-Report

The Dental Fear Survey (DFS; Kleinknecht et al., 1973) consists of 20 items (scored 1-5; total range 20-100) that assess dental fear on several dimensions including avoidance behaviors, physiological sensations, and anxious feelings.

The Fear of Dental Pain (FDP; van Wijk & Hoogstraten, 2003) questionnaire consists of 18 items (scored 1-5; total range 18-90) that assess fear of experiencing pain associated with various dental procedures, ranging from having a toothache to having the gums burned away.

The state and trait portions of the State-Trait Anxiety Inventory (STAI; Spielberger, 1983), as well as the Beck Depression Inventory (BDI-II; Beck, Steer and Brown, 1996) were used to assess possible differences in anxiety and depression between the two dental fear groups.

After participants were removed from the scanner, they rated the unpleasantness of shock anticipation and reactions to neutral and unpleasant picture content on a 7-point scale ranging from unpleasant (1) to pleasant (7).

Procedure

After entering the scanner, the shock electrode was attached to the participant’s ankle and the shock contingencies were repeated to the participant. Participants were

instructed to view each stimulus while it was on the screen and to maintain their gaze on a centrally presented fixation cross.

Data Acquisition and Reduction

Skin conductance

Skin conductance was recorded during scanning using two large Ag/Ag-CL electrodes attached to the left hypothenar eminence of the left palm. A low, constant voltage AC excitation (22 mV at 75 Hz) was supplied to one of the electrodes using a Powerlab ML116 –GSR amplifier. Skin conductance levels were sampled continuously at 1000 Hz using a laptop computer running Chart software (v 5.5.1, ADInstruments, Inc., Colorado Springs, CO). Activity was filtered offline in MATLAB (Mathworks, Inc., Natick, MA) using a 3 Hz low pass filter, then averaged into half-second bins, baseline deviated from mean activity 1 s prior to cue onset, and averaged over the 12 s cue period for each trial and condition.

Functional magnetic resonance imaging

A T1-weighted anatomical volume was acquired using a Siemens 3T *Allegra* MR scanner. A total of 546 functional volumes (50 coronal slices, 2.5 mm thick, .5 mm gap) were collected using a T2* weighted echo planar imaging sequence (3 s TR, 35 ms TE, 160 mm FOV, 64 x 64 acquisition matrix).

Functional data were slice-time adjusted, motion corrected, spatially smoothed (5 mm FWHM Gaussian kernel), and converted to percent blood oxygen level-dependent (BOLD) signal change using the Analysis of Functional Neuroimages software (AFNI; Cox, 1996). Hemodynamic responses were deconvolved using a multiple linear regression model that estimated event-related signal change as a linear combination of 8 uniform B-spline basis functions in addition to regressors of non-interest that modeled

motion residuals and baseline drift. For each voxel and for each condition, this generated a time series of beta coefficients equal in length to a single trial (24 s). Resultant time series were spatially normalized (Talairach and Tournoux, 1988), and resampled to a 2.5 mm isotropic voxel size.

Data Analysis

Using participants' average percent BOLD signal change from 6-15 s post cue onset (to account for hemodynamic delay during the 12 s cue period), two mixed-effect model analyses of variance (ANOVA) were used to separately identify regions 1) activated during threat compared to safe periods when cues were presented alone, and 2) when viewing unpleasant compared to neutral pictures in the context of safety. Each contrast included phase (before/after shock exposure), and cue (threat, safe) or picture valence (unpleasant, neutral) as within-participant factors. Dental fear was not included as a between participant factor in these contrasts to avoid biasing subsequent region of interest analyses.

To determine critical *F*-statistic values for each contrast that corrected for multiple comparisons across the volume, non-parametric permutation tests were computed following recommended guidelines (Nichols & Holmes, 2002). In each permutation test, labels coding for the relevant variable (e.g., cue or picture valence) were randomly reassigned within participants and checked for independence from previous permutation orders. An *F*-statistic was then generated at each voxel and a Gaussian function fit to their distribution over the entire brain. The value of the *F*-statistic at the 99.9 percentile of the fitted Gaussian distribution was selected to form a permutation distribution based on 10,000 randomizations. The resulting absolute thresholds that corrected for multiple comparisons at $p < .05$ were $F(1,31) = 12.15$ ($p < 0.0019$ uncorrected) for the contrast

of threat and safe cues presented without pictures, and $F(1,31) = 13.63$ ($p < 0.0011$ uncorrected) for the contrast of unpleasant and neutral pictures viewed during safe periods.

Functional regions of interest were specified based on the union of the clusters activated in the two, whole-brain contrasts described above. Regions were then grouped into three networks comprised of brains regions a) exclusively activated under threat of shock b) exclusively activated during aversive perception, c) or that were activated in both contexts. These networks were based on effect sizes computed for each contrast and region. Generalized omega-squared (ω_G^2; Olejnik & Algina, 2003) was used to estimate effect sizes and describes the percent variance accounted for by an experimental factor. A region was considered activated in either contrast if its associated ω_G^2 under that contrast exceeded .09 (a medium sized effect; Cohen, 1988). Classification was based on ω_G^2 rather than *t*-statistics as this procedure limits the influence of Type I and Type II error, yields a valid metric that can be compared across regions and studies, is an appropriate effect size estimate for small samples (Olejnik & Algina, 2003), and can be meaningfully related to estimates of effect magnitude as defined by Cohen (1988).

For each network, mixed-model ANOVAs were then conducted on BOLD activity using all the data, including the variables of Cue (threat, safe), Content (no picture, neutral, unpleasant), Region, and Phase (before, after shock exposure), as within-participant factors, and Fear (high, low dental fear) as a between-participant factor. In these analyses, variables in the full design were assessed for their effects in each of the

three identified networks. Sphericity violations and all pair-wise comparisons were controlled using the Greenhouse-Geisser and Bonferroni corrections, respectively.

Skin conductance activity was analyzed using a similar ANOVA model that included the variables of Cue, Content, and Phase as within-participant factors, and Fear as a between-participant factor.

Results

Self-Report

Questionnaires

Questionnaire scores for the low and high fear groups are listed in Table 3-1. As expected, high dental fear participants scored higher on the Dental Fear Survey than low fear participants ($F(1,30) = 89.46$, $p < .0001$). Compared to the low fear group, the high dental fear group also reported a greater fear of dental pain on the Fear of Dental Pain questionnaire ($F(1,30) = 11.17$, $p < .005$). Groups did not differ in their reported experience of depressive symptoms, trait anxiety, or state anxiety using the Beck Depression Inventory and Spielberg State-Trait Anxiety Inventory (all $F < 1$).

Emotional ratings

Participants rated shock anticipation as more unpleasant before shock exposure compared to after shock exposure ($F(1,30) = 15.2$, $p < .0001$), and in comparison to actual shock ($F(1,30) = 23.6$, $p < .0001$). As expected, unpleasant pictures were rated as more unpleasant than neutral pictures, $F(1,30) = 110.5$, $p < .0001$). Unpleasantness ratings for shock anticipation and picture contents did not differ as a function of dental fear.

Skin Conductance

Table 3-2 lists mean skin conductance change during threat and safe periods for cues presented with and without pictures, as a function of dental fear. Threat cues elicited larger increases in skin conductance activity compared to safe cues ($F(1,30) = 6.21$, $p < .05$), when presented alone ($t(31) = 2.1$, $p < .05$), or when presented with a neutral ($t = 2.3$, $p < .05$) or unpleasant picture ($t = 2.1$, $p < .05$; Figure 3-1). The magnitude of skin conductance change to threat and safe cues did not differ for cues presented with or without a picture. Overall, viewing unpleasant pictures elicited greater sympathetic activity than viewing neutral scenes ($t = 2.2$, $p < .05$). These effects were not modulated by dental fear or shock exposure.

Neuroimaging

Whole-brain analyses

Whole-brain analyses were conducted that compared at each voxel, mean BOLD activity during threat and safe cues presented without pictures, and during the perception of unpleasant and neutral pictures viewed during safe periods. Table 3-3 lists peak activation coordinates and associated statistics for regions activated in either contrast.

When presented without a picture, threat cues prompted larger BOLD signal increases compared to safe cues bilaterally in the superior medial frontal gyrus (BA 6/8), anterior insula, inferior frontal gyrus—with separate activation foci in the pars orbitalis (BA 45/47) and pars triangularis (BA 44) subdivisions—and medial portion of the middle temporal gyrus (BA 21). Increased activation under threat of shock was also evident bilaterally in the supramarginal gyrus (BA 40), posterior cerebellum, and anterior

superior temporal gyrus. Right-lateralized activations were present in the lateral superior frontal gyrus, ventral anterior thalamus, and caudate nucleus.

Unpleasant pictures elicited greater signal change compared to neutral pictures throughout ventral visual and dorsal parietal cortex, with focal activations located in the fusiform gyrus, bilateral middle occipital cortex, posterior middle temporal gyrus and superior parietal lobule. In addition to enhanced activation of the visual and parietal cortices, unpleasant pictures also prompted greater activation bilaterally in the amygdala and middle frontal gyrus.

In addition to these regions that uniquely showed heightened activation during unpleasant picture processing, several additional regions which were activated by threat cues, also showed increased activation during unpleasant versus neutral pictures: the superior medial frontal gyrus, inferior frontal gyrus, supramarginal gyrus, medial middle temporal gyrus, and anterior insula (at a reduced threshold, $p = .0062$ uncorrected).

Regions identified in the cue and picture valence contrasts described above, were partitioned into 3 functional networks based on generalized omega-squared (ω_G^2) effect size statistics. Figure 3-2 illustrates grouping of the activated regions into 3 functional networks: regions uniquely activated under threat of shock (*"Threat of Shock"*), regions uniquely activated during aversive perception (*"Aversive Perception")*, and regions activated during both threat of shock and aversive perception (*"Overlap"*). Medium sized or larger effects ($\omega_G^2 > .09$) were specific to the contrast of threat and safe cues, presented without a picture, for the following three regions: lateral superior fontal gyrus, ventral anterior thalamus, and the dorsolateral head of the caudate. Medium sized or larger effects were limited to the contrast of unpleasant and neutral

pictures, viewed under safety, for the following six regions: amygdala, fusiform gyrus, middle occipital gyrus, posterior middle temporal gyrus, superior parietal lobule, and middle frontal gyrus. Medium sized or larger effects were evident for both contrasts in six regions: anterior insula, superior medial frontal gyrus, inferior frontal gyrus, medial middle temporal gyrus, and supramarginal gyrus.

Multivariate region of interest analyses of functional networks

Separate mixed-model ANOVAs were conducted on the BOLD activity in each of the three functional networks to determine (a) if the effect that defined each network varied with the context, (b) if the activity in each network was modulated by dental fear (i.e., fear of pain), (c) if there was a cumulative effect on BOLD signal change when unpleasant pictures were viewed under threat of shock.

Neural activity unique to threat of shock

Figure 3-3 A shows the overall pattern of BOLD activity in the threat of shock network, which includes the three regions that were uniquely activated under threat of shock: superior frontal gyrus (Figure 3-3 B), thalamus, and caudate. Overall, threat cues elicited greater signal increases than safe cues ($F(1,30) = 15.26$, $p < .0001$). As expected activity in this network was enhanced during threat compared to safe cues presented without pictures ($t(31) = 4.59$, $p < .0001$), but more importantly, activation of this network was similarly heightened when threat cues were presented with a picture ($t = 3.24$, $p < .05$). The amount of BOLD activity elicited during threat periods in this network did not differ between threat cues presented with or without pictures (Cue x Content, $F < 1$, *ns*). An interaction of Region, Cue, and Phase ($F(2,60) = 4.98$, $p < .05$) indicated that enhanced activation in the superior frontal gyrus during threat cues declined following shock exposure ($t = 3.18$, $p < .005$) and no longer differed from

activity elicited by safe cues ($t < 1$, *ns*). Dental fear did not modulate any of these effects.

Neural activity unique to aversive perception

Figure 3-4 A illustrates the effect of picture content on BOLD activity, averaged across the six regions uniquely activated during aversive perception: amygdala, inferotemporal cortex, middle occipital gyrus, posterior middle temporal gyrus, superior parietal lobule, and middle frontal gyrus. Group-averaged, event-related time courses of BOLD signal change in the amygdala and inferotemporal cortex are illustrated in Figure 3-4 B.

Overall, viewing unpleasant pictures consistently elicited larger signal increases relative to neutral pictures (Content, $F(2,60) = 127.67$, $p < .0001$). Greater activity in this network was expected in this network when unpleasant pictures were viewed during safe periods ($t = 4.83$, $p < .0001$). More importantly increased activation of this network was also found when unpleasant, compared to neutral, pictures were viewed under threat of shock ($t(31) = 4.79$, $p < .0001$). Not surprisingly, cues presented in the context of pictures elicited greater activity in this network than cues presented without pictures ($F(1,31) = 112.02$, $p < .0001$). However, whether a cue signaled threat or safety did not impact BOLD activation in this network (Cue, $F < 1$, *ns*).

An interaction between Region and Content ($F(10,300) = 51.06$, $p < .0001$) indicated that the difference in activity between unpleasant and neutral pictures was larger in visual cortex (e.g., inferotemporal cortex, middle occipital gyrus, and posterior middle temporal gyrus) than in other regions (e.g., superior parietal lobule, amygdala, and middle frontal gyrus ($F(5,155) = 10.40$, $p < .0001$). Across all six regions, there was

an overall decline in BOLD activity after shock exposure (Phase, $F(1,30) = 8.24$, $p < .005$).

Neural activity common to threat of shock and aversive perception

Figure 3-5 A depicts mean BOLD activity in the network activated during threat of shock and aversive perception: anterior insula, superior medial frontal gyrus, inferior frontal gyrus, supramarginal gyrus, medial middle temporal gyrus, and posterior cerebellum. Figure 3-5 B illustrates functional activity in regions hypothesized to be activated in both contexts.

An interaction of Cue and Content ($F(1,30) = 6.74$, $p < .05$, linear contrast), indicated that the difference in signal change during threat and safe periods was affected by picture content. Threat cues elicited greater activity relative to safe cues when presented alone ($t(31) = 5.15$, $p < .05$) or with a neutral picture ($t = 2.25$, $p < .05$). However, there was no additional increase in signal change when unpleasant pictures were viewed under threat of shock, compared to when unpleasant pictures were viewed during safe periods ($t = 1.12$, *ns*). In fact, activity in these regions for unpleasant pictures viewed under safety was equivalent to that elicited by threat cues of any sort—whether presented alone, or with a neutral or unpleasant picture ($F(3,93) < 1$, *ns*). Also, while signal increases were larger during unpleasant compared to neutral pictures viewed under safety ($t = 2.55$, $p < .05$), picture valence no longer modulated signal change when pictures were viewed under threat of shock ($t = 1.04$, *ns*).

Reported dental fear affected activity in this network (Dental Fear x Cue, $F(1,30) = 4.173$, $p < .05$). In participants reporting high dental fear, threat cues elicited larger overall signal increases in this network compared to low fear participants ($t(31) = 1.95$, $p < .05$; Figure 3-6 A), especially when threat cues were presented alone ($t = 2.39$, p <

.05). When questionnaire scores were ranked and treated as continuous variables in analyses that predicted mean signal change in this network during threat periods (Figure 3-6 B), results confirmed larger signal increases under threat of shock as function of dental fear (DFS, $r = .46$, $p < .005$) and fear of dental pain (FDP, $r = .56$, $p < .0005$; Figure 3-5 B). Ranked questionnaire scores also predicted the difference in activity between threat and safe periods (DFS, $r = .46$, $p < .01$; FDP, $r = .45$, $p < .01$), averaged across regions in the network.

To further explore these effects, signal change during threat and safe periods were additionally compared between the high and low dental fear groups for each region in this network. Participants reporting high, compared to low, dental fear showed larger signal increases during threat in inferior frontal gyrus ($t(31) = 2.92$, $p < .01$), superior medial frontal gyrus ($t = 2.4$, $p < .05$), supramarginal gyrus ($t = 2.0$, $p < .05$), and anterior insula ($t = 1.7$, $p < .05$). In addition, safe cues prompted less activity in high compared to low fear participants in medial middle temporal gyrus ($t = 1.88$, $p < .05$), and posterior cerebellum ($t = 2.39$, $p < .05$).

An interaction of Phase and Cue ($F(1,30) = 8.27$, $p < .01$) indicated that increased activation during threat relative to safe periods was modulated by shock exposure. While threat cues elicited greater signal change than safe cues both before ($t(31) = 4.18$, $p < .0001$) and after shock exposure ($t = 2.23$, $p < .05$), threat cues generally elicited less activity in this network following shock exposure ($t = -4.14$, $p < .0001$). Exposure related declines in threat-related activity were largest in the superior medial frontal gyrus and smallest in the posterior cerebellum, compared to all other regions in the network (Region x Phase x Cue, $F(6,180) = 3.25$, $p < .01$). An interaction

of Region and Content ($F(12, 76) = 3.77$, $p < .005$) indicated that overall, BOLD activity prompted by unpleasant pictures was largest in the posterior cerebellum, inferior frontal gyrus, and superior medial frontal gyrus compared to responses elicited in the anterior insula and medial temporal gyrus.

Discussion

The neural circuits mediating brain activity during threat of shock and aversive perception were compared to determine common and distinct brain circuits activated in each context. Regions uniquely activated during threat of shock included the superior frontal gyrus, the caudate, and the thalamus. In contrast, unpleasant picture processing uniquely activated the amygdala, middle occipital and fusiform gyrus, superior parietal visual areas, and right middle frontal gyrus. Regions that were activated during both threat of shock and aversive perception included the anterior insula, superior medial frontal gyrus, ventrolateral inferior frontal gyrus, middle temporal gyrus, supramarginal gyrus, caudate, and posterior cerebellum. Together these results suggest that threat of shock and processing of unpleasant pictures engage common and distinct neural structures and circuits.

Threat of Shock Uniquely Activates Regions Involved In Aversive Learning.

Threat of shock specifically heightened activation in an anterolateral portion of the superior frontal gyrus extending into the middle frontal gyrus, the dorsolateral head of the caudate, and thalamus. These regions comprise a classic basal ganglia thalamocortical loop with the prefrontal cortex (Alexander, Delong & Strick, 1986; Middleton & Strick, 2000), thought to underlie various aspects of cognition (Fuster, 2008). Activation of this circuit when anticipating shock might reflect working memory or learning processes associated with determining whether a cue signaled threat or safety.

Consistent with this conceptualization, anterior frontal cortex activity is consistently activated during learning of abstract rules and categorization parameters (Strange, Henson, Friston & Dolan, 2001). More related to the current study, during delay and trace fear conditioning, awareness of the reinforcement contingencies and shock expectancy ratings are associated with heightened bilateral activation of the anterior middle frontal gyrus (Carter et al., 2006; Knight et al., 2004). Interestingly, expectancy related activation in anterior frontal cortex is maximal during the trace interval separating presentation of a conditioned and unconditioned stimulus during trace conditioning (Knight et al., 2004). This is notable, since in the same way that threat of shock facilitates fear conditioning compared to classical conditioning (Hughdal, 1978), instructing participants about the cue contingencies prior to trace conditioning facilitates the acquisition of conditioned responses compared to an uninstructed group (Clark, Mann & Squire, 2002). Instructions may therefore facilitate encoding of the threat and safety contingencies in anterolateral prefrontal cortex. Furthermore, the coordinates reported here for activation of the superior frontal gyrus (which extended bilaterally into the adjacent anterior middle frontal gyrus) are, in fact, near those reported in these prior studies (Carter et al., 2006; Knight et al., 2004; Strange et al., 2001).

Further supporting the view that superior frontal gyrus represents cue associations is that threat related activation in the superior frontal gyrus was reduced following shock exposure. This decline in activation of the superior frontal gyrus mirrored the change in participants' aversiveness ratings before and after exposure to a single mild shock. Together the present results, along with prior evidence linking the superior frontal gyrus

to contingency awareness during fear conditioning, and categorization learning, suggest this region encodes the instructed threat contingencies.

The dorsolateral caudate, or dorsal striatum, is also implicated in associative learning, both appetitive and aversive (Delgado, Li, Schiller & Phelps, 2008; Balleine, Delgado, & Hikosaka, 2007). It is particularly engaged in learning contexts where there is uncertainty about whether a cue will be followed by aversive stimulation (e.g under partial reinforcement, fear extinction, fear reversal). Considering that in Experiment 2 participants are instructed about the threat contingencies but are given no additional information about the intensity, timing, or probability of shock, this might explain the unique activation of the caudate during threat of shock. Unlike the superior frontal gyrus, threat-related activation in the caudate did not habituate following exposure to a single mild aversive shock. Perhaps increased caudate activation during threat periods relates more to uncertainty about whether or not shock will occur. This information may then be integrated into the basal ganglia cortico-thalamic circuit identified here. Network coordination in this manner might maintain vigilance to threat despite a change in the expectancy of shock, either in probability or in the intensity of exposure.

Aversive Perception Uniquely Activates Amygdala and Visual Cortex

Viewing unpleasant pictures during safe periods prompted larger signal increases in the amygdala and across multiple regions in ventral visual cortex and dorsal parietal cortex, compared to neutral pictures. This pattern of activation is reliably found during the perception of emotionally arousing pleasant and unpleasant pictures (Bradley, Sabatinelli, Lang, Fitzsimmons, King, & Desai, 2003; Lang, Bradley, Fitzsimmons, Cuthbert, Scott, Moulder, & Nangia, 1998; Sabatinelli et al., 2005; Sabatinelli et al., 2007) and interpreted as evidence of re-entrant feedback between the amygdala and

visual cortex, leading to enhanced perceptual processing and motivated attention to picture contents (Sabatinelli et al., 2009, 2005; Amaral & Price, 1984; Spiegler & Mishkin, 1981).

In Experiment 2, similar patterns of activation in the amygdala and visual cortex were found whether unpleasant pictures were viewed under threat of shock or safety. Essentially, threat of shock did not disrupt the motivational activation observed under safe conditions. This substantiates the finding that emotional modulation of the late positive potential during emotional perception is unaffected when emotional and neutral pictures are viewed under threat of shock or safety (Bublatzky, Flaisch, Stockburger, Schmalze & Schupp, 2010). It seems then that emotionally arousing, unpleasant pictures drive motivational and visual processing independent of the context in which they are viewed.

Threat of Shock and Aversive Perception Activate a Common Network

Several regions that showed heightened activation during threat of shock were also found to show increased activation during aversive perception (e.g., the *"Overlap"* network). This confirms the qualitative overlap in the regions reported to be active in previous studies, which separately investigated threat of shock and aversive perception. Prior studies reporting increased activation in one or more of these regions include those investigating the anticipation and receipt of monetary reward (Knutson & Greer, 2008), perception of pleasant pictures (Scharpf, Wendt, Lotze & Hamm, 2010; Sabatinelli et al., 2007), and imagery of pleasant and unpleasant scenes (Costa, Lang, Sabatinelli, Versace & Bradley, 2010; Sabatinelli, Lang, Bradley & Flaisch, 2006). This suggests that activation of these regions—especially the anterior insula and superior medial frontal gyrus—is not limited to aversive contexts, but rather that these regions

may be engaged in terms of motivational significance (Ferrari, Codispoti, Bradley & Lang, 2011).

Interestingly, signal increases in this network when unpleasant pictures were viewed during safe periods did not differ from the amount of signal change elicited by threat cues, either when presented alone, or with a neutral or unpleasant picture. In addition, BOLD activity during threat periods did not differ based on whether pictures were unpleasant or neutral. Simply put, this circuit reacted equivalently to all "bad" things. Moreover, participants reporting high dental fear showed increased activation in this network, specifically during threat periods, suggesting that activation in this network does reflect, and might mediate, the affective component of threat of shock.

The lack of additive effects on signal change when unpleasant pictures were viewed under threat of shock indicates these regions do not respond linearly during highly arousing, or multiple aversive events. Considering that these same regions are also activated in appetitive contexts, it may be the case that activation of this network generally reflects whether a stimulus is appetitive or aversive, rather than its intensity. However, our enthusiasm for this hypothesis is tempered by the fact that previous studies have correlated activation of the anterior insula, superior medial frontal gyrus, and inferior frontal gyrus with ratings of emotional arousal (Lewis, Critchley, Rotshtein & Dolan, 2007; Phan, Taylor, Welsh, Ho, Britton & Liberzon, 2004), pain intensity (Koyama, McHaffie, Laurienti & Coghill, 2005), as well as increases in skin conductance (Critchley, Elliot, Mathias & Dolan, 2000; Critchley, 2005)—which is itself strongly correlated with arousal ratings during emotional perception (Bradley et al., 2001).

Another possibility is that functional activation of these structures is saturated, similar to fear-induced saturation of amygdala responses in rodents, when multiple conditioned stimuli are presented simultaneously (Walker & Davis, 2002). It is important to note though, that observed effects are likely not related to a saturation of the BOLD signal itself, since examination of regional BOLD waveforms indicated that the hemodynamic signal increased throughout the cue period. Rather the effects seen here parallel a similar lack of additivity in startle reflex potentiation when unpleasant pictures cued threat of shock or safety (Bradley et al., 2005). Notably, a recent study found a linear increase in signal change in the inferior frontal gyrus and anterior insula corresponding to the intensity of the shock that was anticipated, ranging from medium to strong (Drabant et al., 2010). Thus, it seems unlikely that a ceiling effect obscured further increases in BOLD signal change in this network when participants viewed unpleasant pictures under threat of shock.

A plausible, though more daunting explanation is that neural activity in these regions occurs in such a way, that conventional neuroimaging techniques are limited in their ability to detect additive effects. If this is true more sophisticated analyses and experimental designs might be needed. For instance, estimates of functional or effective connectivity between all regions in the network, or between specific pairs of regions, might reveal that the defensive system is increasingly activated with exposure to multiple aversive stimuli. Future studies will need to keep these possibilities in mind to determine how the brain regions identified here support defensive and appetitive engagement.

Table 3-1. Mean questionnaire scores and rated unpleasantness of shock anticipation and shock exposure as a function of dental fear.

	High fear	Low fear
Dental fear survey (DFS)	72.8 (8.4) [a]	42.8 (8.5)
Fear of dental pain (FDP)	71.7 (3.1) [a]	57.2 (2.6)
State Anxiety (STAI-S)	38.8 (3.0)	35.9 (2.3)
Trait Anxiety (STAI-T)	39.9 (8.5)	35.4 (9.9)
Beck Depression Inventory (BDI-II)	7.4 (1.3)	7.4 (1.3)
Unpleasantness of shock anticipation [b]	3.2 (.27)	3.3 (.26)
Unpleasantness of shock exposure [b]	3.7 (.52)	3.7 (.41)

[a] Between-group comparison significant at $p < .05$.
[b] The ratings scale ranged from 1 = unpleasant to 7 = pleasant

Table 3-2. Skin conductance change (µ-siemens) to threat and safe cues presented with and without pictures as a function of dental fear.

	High dental fear		Low dental fear	
Picture content	Threat	Safe	Threat	Safe
No picture	.17 (.08)	-.06 (.04)	.07 (.04)	-.03 (.04)
Neutral	.08 (.07)	-.03 (.04)	.05 (.06)	-.07 (.03)
Unpleasant	.16 (.09)	-.01 (.02)	.08 (.07)	-.02 (.02)
Mean	.14 (.08)	-.03 (.02)	.07 (.02)	-.04 (.02)

Table 3-3. Peak activation coordinates and statistics for regions activated when comparing threat versus safe cues and unpleasant versus neutral pictures.

	Contrast									
	Cue Alone: Threat > Safe					Safety: Unpleasant > Neutral				
Region	x	y	z	*t* [a]	μL [b]	x	y	z	*t* [a]	μL [b]
Superior medial frontal gyrus	-1	42	34	5.0	688	-1	37	44	4.4	156
	4	42	34	6.5	1188	2	37	44	5.1	438
Inferior frontal gyrus (orb.)	-33	24	-3	6.0	625	-33	22	-11	4.0	375
	32	22	-1	5.7	766	54	27	-1	3.7	141
Middle temporal gyrus (med.)	-48	-28	-1	5.6	281					
	59	-41	2	6.3	984	49	-46	-3	3.4	156
Supramarginal gyrus	-56	-46	24	5.6	1078	-61	-51	19	4.0	16
	62	-48	22	4.9	266	57	-43	22	4.6	156
Cerebellum (post.)	-26	-66	-31	6.1	328	-26	-58	-38	4.9	250
	19	-68	-31	5.1	219	4	-66	-21	5.0	31
Anterior insula [c]	-31	22	4	5.8	516	-33	24	2	2.3	32
	32	17	-6	4.1	78	32	19	-8	2.3	47
Inferior frontal gyrus (tri.)	-44	17	9	6.7	859					
	42	22	7	6.1	703					
Superior frontal gyrus	22	52	29	5.8	188					
Caudate	14	4	17	4.3	94					
Thalamus	-8	-7	7	4.0	47					
	7	-1	9	3.6	16					
Parahippocampal gyrus	-31	-36	-6	-3.8	47					
	29	-31	-9	-4.9	391					
Paracentral lobule	-1	-33	52	-3.5	63					
Postcentral gyrus	24	-36	57	-5.0	172					
Amygdala						-23	-1	-11	4.8	78
						29	-3	-13	4.3	63
Middle occipital gyrus						-48	-76	2	8.0	3219
						47	-61	-3	7.3	5094
Fusiform gyrus						-48	-73	-1	7.5	4328
						42	-43	-13	8.5	5422
Superior parietal lobule						-31	-61	49	5.1	266
						29	-58	49	7.0	1141
Middle temporal gyrus (post.)						-53	-68	7	6.9	2734
						54	-66	7	7.0	4031
Middle frontal gyrus						54	27	12	5.5	594

Note. L = left; R = right; orb = orbitalis division of the inferior frontal gyrus; tri = triangularis division of the inferior frontal gyrus; ant/med/post = anterior/medial/posterior aspect of the referenced region.

[a] Significant at $p < .05$ corrected

[b] Cluster size computed using 2.5 mm^3 voxel.

[c] Significant at $p < .01$ uncorrected for the contrast of unpleasant and neutral pictures.

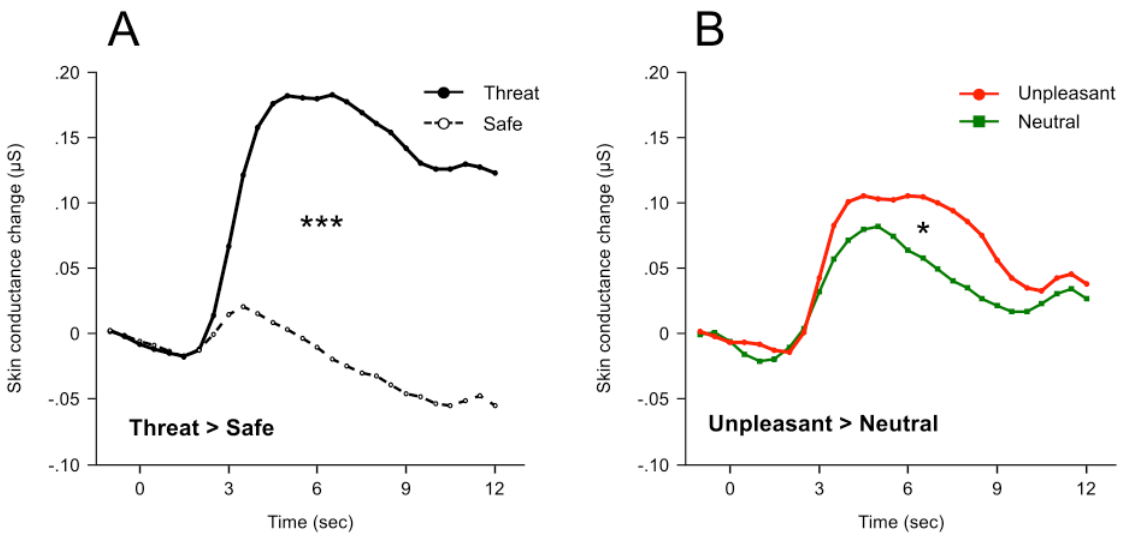

Figure 3-1. Threat cues and unpleasant pictures heighten skin conductance activity. A) For threat and safe cues skin conductance change is averaged over cues presented with and without pictures. B) For unpleasant and neutral pictures skin conductance change is averaged over pictures presented during threat and safe periods.

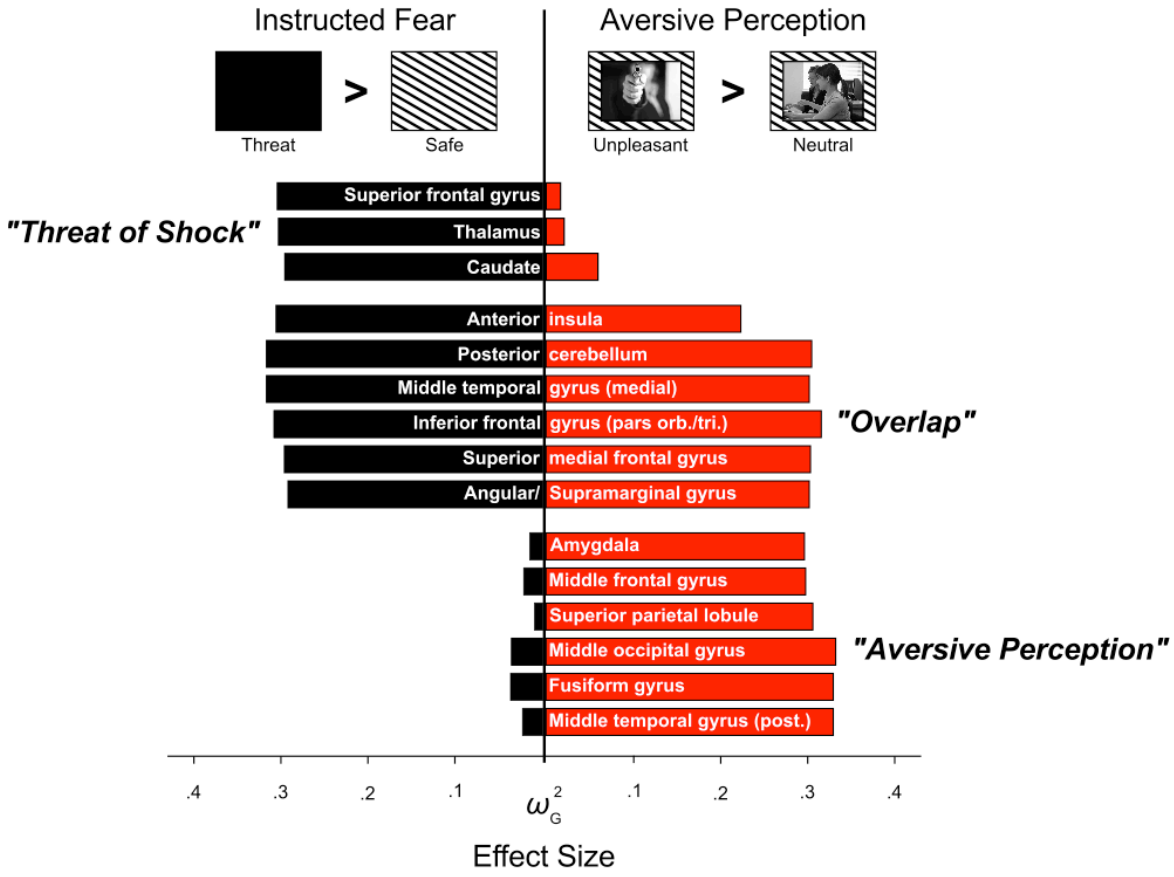

Figure 3-2. Effect sizes and functional groupings of regions activated during threat of shock, aversive perception, or in both contexts. The left-hand column shows effect sizes for comparisons of signal change during threat versus safe periods, when cues were presented without a picture. The right-hand column shows effect sizes for comparisons of signal change during unpleasant versus neutral pictures, when the colored frame surrounding a picture signaled safety.

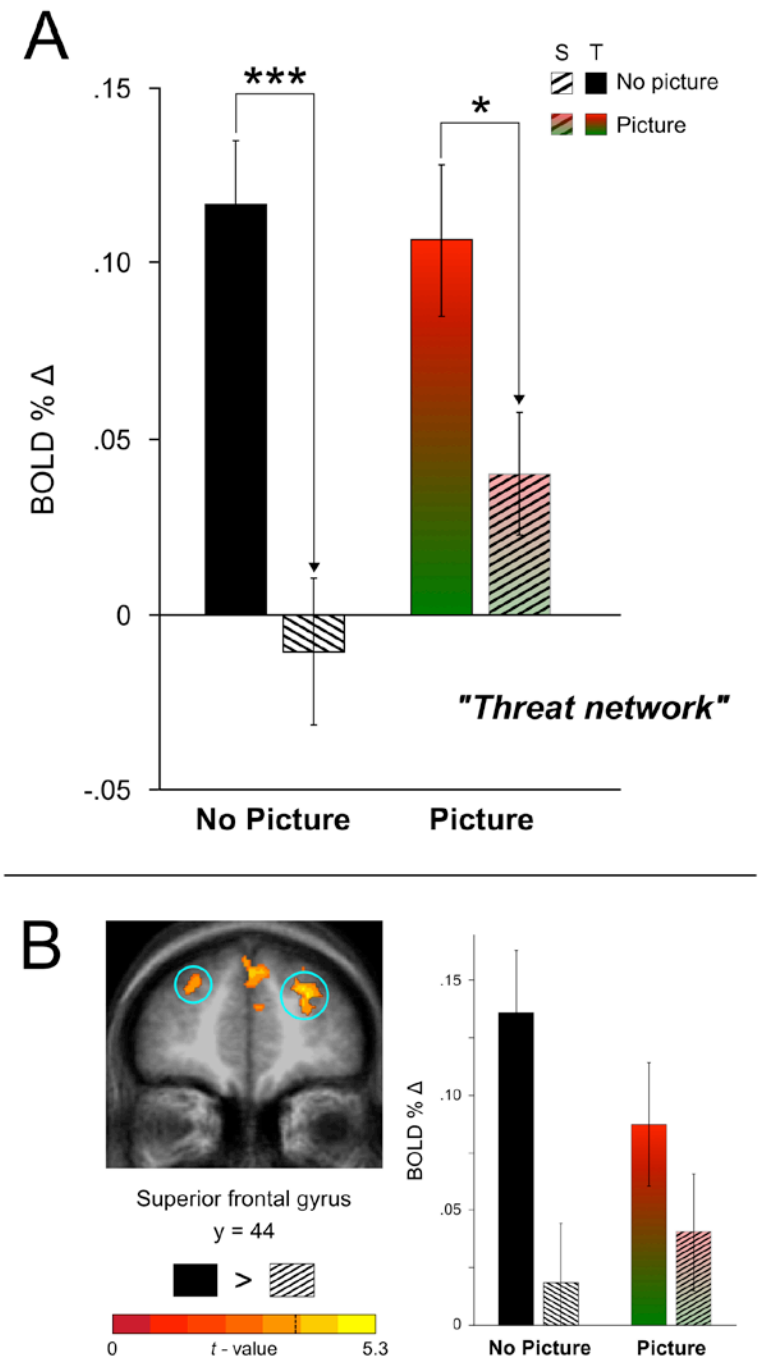

Figure 3-3. Regions uniquely activated during threat of shock in the presence or absence of a picture. A) Mean BOLD activity averaged across all regions in the network. B) Mean signal change in the superior frontal gyrus during threat and safe cues presented with or without a picture.

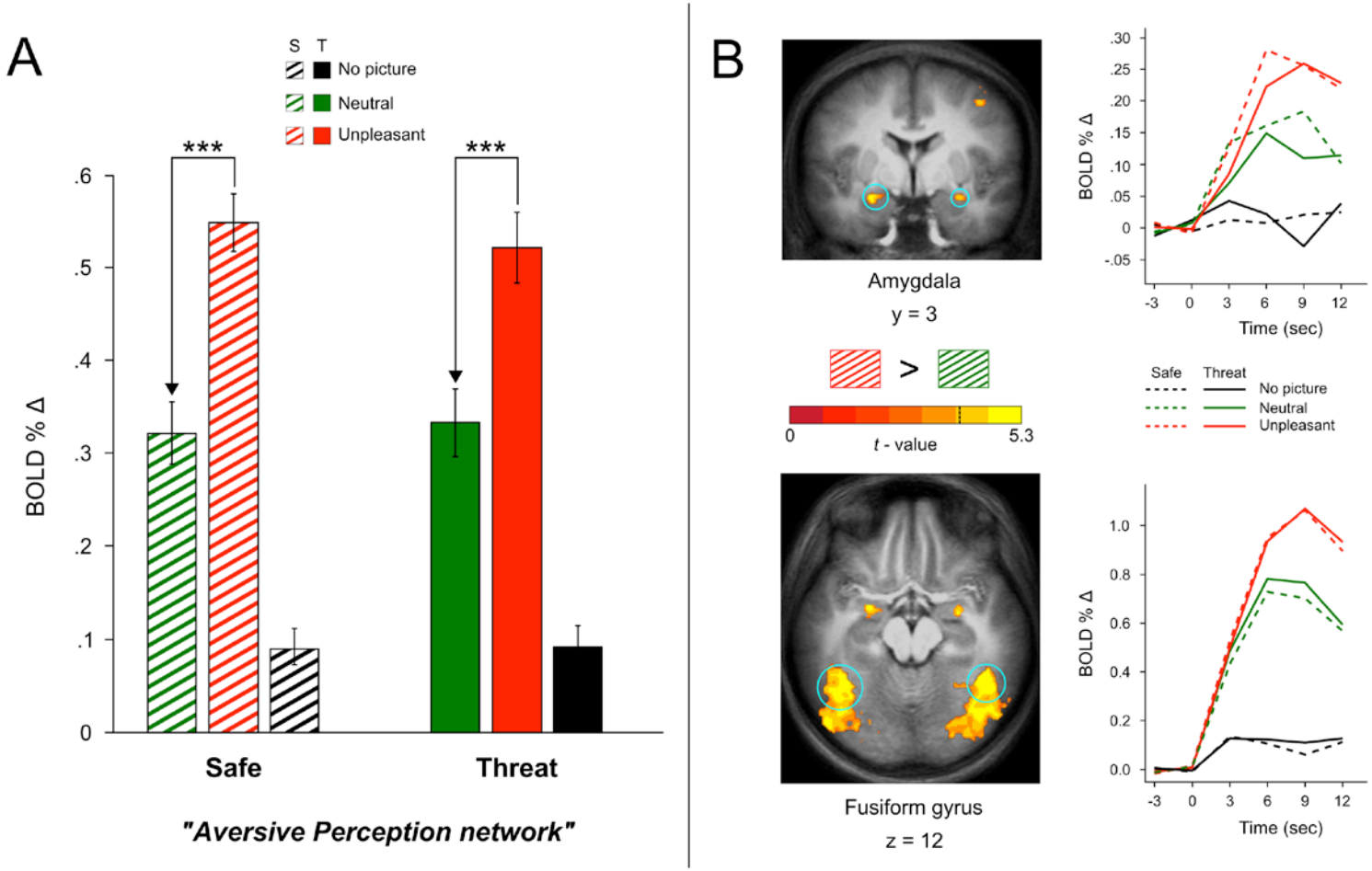

Figure 3-4. Regions uniquely activated during aversive perception are similarly activated when unpleasant and neutral pictures are viewed under threat of shock or safety. A) Mean BOLD activity averaged across all regions in the network. B) Average time course of activity in the amygdala and fusiform gyrus to unpleasant and neutral pictures presented during threat or safe periods.

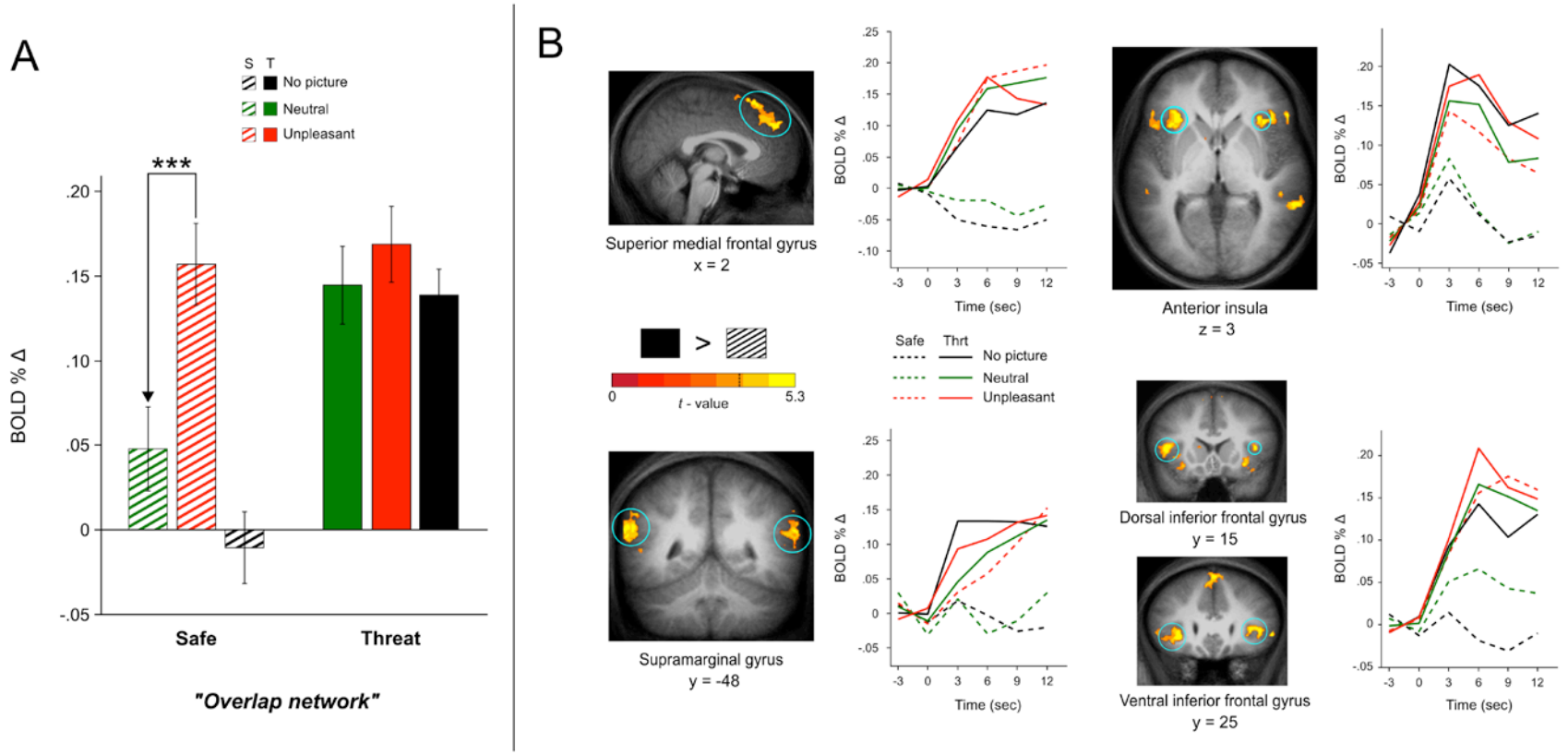

Figure 3-5. Viewing unpleasant pictures in a threatening context does not have a cumulative effect on signal change in regions activated both during threat of shock and aversive perception. A) Mean BOLD activity averaged across all regions in the network. B) Average time course of activity in regions hypothesized to be activated in both contexts during threat and safe cues, presented with or without affective pictures.

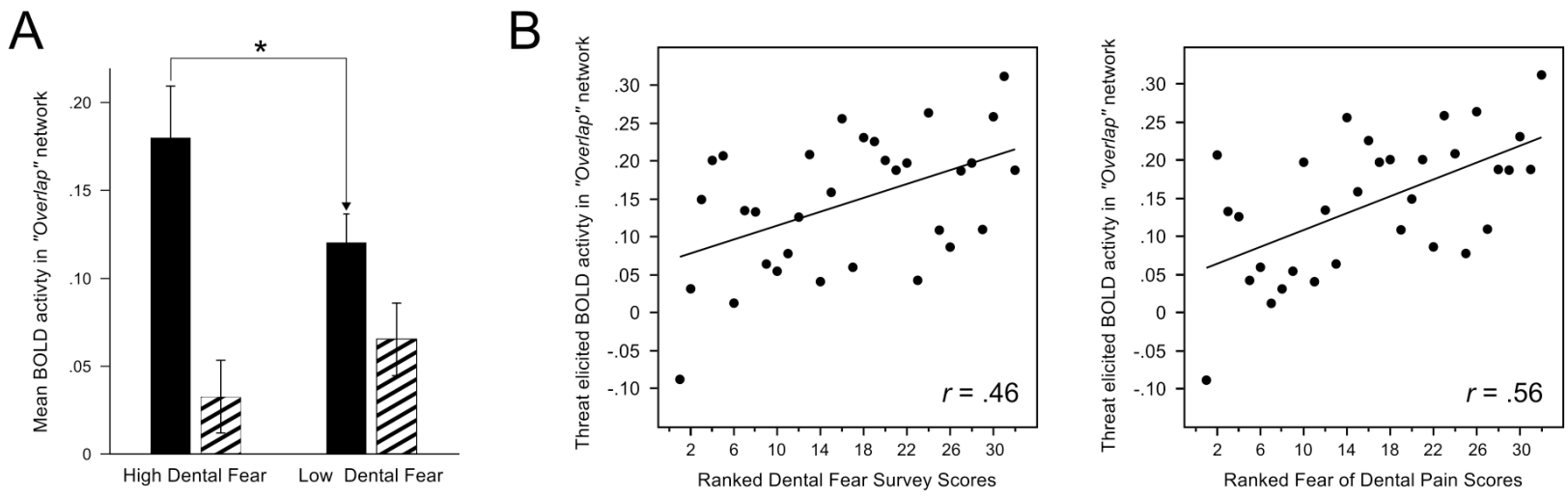

Figure 3-6. Under threat of shock, dental fear is associated with heightened activation of the network activated both during threat of shock and aversive perception. To obtain mean BOLD activity during threat and safe periods in the *"Overlap"* network, signal change was first averaged over cues presented with and without pictures and then averaged across the six regions. A) Activity during threat and safe periods in the high and low dental fear groups. B) Scatter plots show the correlation between dental fear and fear of dental pain (questionnaire scores are ranked in ascending order) with mean activity elicited by threat cues.

CHAPTER 4
GENERAL DISCUSSION

Taken together, the results of Experiments 1 and 2 elucidate how language can control defensive reactions, and identify the neural circuits that support this form of defensive learning. Specifically, simple verbal instructions that describe stimulus associations with shock and safety are effective in engaging the defensive motivational system when a threatening stimulus is presented, and in quickly attenuating its activation—at least as inferred from changes in reflex physiology—when that same cue no longer signals the possibility of shock. These experiments also provide insights into why similar profiles of defensive reactivity are observed during instructed fear and aversive perception—despite paradigmatic differences in terms of amygdala activation—and help to clarify which brain regions likely mediate affective distress when anticipating a painful, aversive stimulus.

These experiments also the lay a foundation for investigating the neural mechanisms underlying the rapid and complete reversal of defensive reactions. Building on the results of both experiments future studies can investigate if there is a parallel rapid adjustment of activity in the commonly activated network identified in Experiment 2, and if this reversal in activity is intrinsic to network itself or if it relies on the activation of additional regions previously implicated in fear inhibition and or serial reversal learning. Going forward, the instructed reversal of fear paradigm will be a productive complement to existing fear conditioning and cognitive emotion regulation protocols, in determining the neural involved in fear inhibition.

Startle Potentiation and the Lack of Amygdala Activation Under Threat of Shock

Increased startle potentiation under threat of shock was initially presumed to reflect heightened activation of the amygdala. This view was based on extensive evidence in animals that fear potentiated startle involved projections of the central nucleus of the amygdala to the nucleus reticularis pontis caudalis (PnC), a primary synapse in the acoustic startle pathway (Davis, 2006; Davis, Gendelman, Tishcler, & Gendelman, 1982), and on an initial neuroimaging study in humans that reported heightened amygdala activation during threat of shock (Phelps et al., 2001). Previous findings of increased startle potentiation and heightened amygdala activation when humans viewed emotionally arousing, unpleasant pictures (Lang, 1995; Sabatinelli et al., 2005, 2007, 2009) also seemed to support the view that increased startle potentiation depended on amygdala activation.

The findings of Experiment 2, however, indicated that amygdala activation did not differ during threat and safe periods, whereas significant amygdala activation was found when viewing unpleasant pictures. A lack of amygdala activation when processing threat cues is consistent with a recent meta-analysis of studies that examined neural activation during threat of shock and found that the amygdala was not reliably activated (Mechias, Etkin & Kalisch, 2010). The authors of this meta-analysis suggested a number of reasons for the poor replication of amygdala activation during threat of shock. One possibility is that imaging at higher field strengths might increase susceptibility related artifacts and prevent reliable measurement of signal change in the amygdala. Experiment 2 addressed this concern by demonstrating at a commonly used, high field strength (3-Tesla), successful imaging of differential amygdala activity to unpleasant

and neutral pictures, while still finding no difference in amygdala activation during threat and safe periods.

Another possibility is that the initial study by Phelps and colleagues (2001) only involved five threat trials and as a result habituation of amygdala activity was minimal—especially in comparison to other threat of shock studies, which typically involved a greater number of threat trials (Mechias, Etkin & Kalisch, 2010). For example, in Experiment 2 there were 32 presentations of a threatening cue. Still even when analyses were confined to the first few trials in Experiment 2, there was no reliable difference in amygdala activation between threat and safe periods. Moreover, previous studies that have reported habituation of amygdala activation during threat of shock have only compared activity relative to baseline and not relative to activity during safe periods (e.g., Butler et al., 2007). While startle potentiation under threat of shock does habituate over time, as seen in Experiment 1 (see Figure 2-2), blink magnitude remains potentiated during threat relative to safe periods throughout the entire experiment, even after amygdala activation is presumed to have habituated. For instance, Bradley and colleagues (2008) found that threat-related startle potentiation was still evident even after sixty-four trials and a half an hour of stimulus presentation, without any reinforcement other than a single mild shock.

How might we account for the increased startle potentiation robustly observed during threat of shock in Experiment 1, given the absence of reliable activation of the amygdala in Experiment 2? Perhaps a parsimonious explanation—without any appeal to methodological differences—is simply that threat of shock paradigms do not involve an acquisition phase with actual pairings of a neutral and unconditioned stimulus (US).

There is extensive evidence in rodents that learned associations between a cue and an aversive US are encoded in the basolateral nucleus (BLA) of the amygdala and that lesions of BLA interfere with the acquisition and expression of fear conditioned responses (Schafe & Ledoux, 2008), including fear-potentiated startle. When language is used to associate a stimulus with a cognitive representation of a feared event, especially one that has yet to be experienced, it is unlikely that this abstract association is represented in the amygdala (Olsson & Phelps, 2007). Rather such an association is more likely encoded in a distributed cortical network that integrates processing of current stimulus features with past experiences, memories, and expectations of the described aversive event (Olsson & Phelps, 2007; Lang, 1979).

Such an explanation might also account for why the amygdala is activated during aversive perception and not during threat of shock. Over time, humans learn about their environment and the stimuli it contains through direct experience, verbal communication symbolic, observation, and sociocultural means. Pictures of mutilated bodies, or scenes of violence, at least partially match representations of similar emotionally important events and stimuli that have entered into long-term memory through these different learning processes. It is theorized (Lang, 1979) that the semantic, perceptual, and response-related properties of stimuli in memory are encoded in distributed associative networks that in part include motivational circuits and structures, such as the amygdala. Viewing unpleasant pictures presumably activates these networks, including activation of the amygdala, and as a result naturally elicits emotional responses.

Moreover, models of visual perception suggest a reentrant organization of the ventral visual system with the amygdala in order to facilitate visual emotional

discrimination. In threat of shock paradigms, threat and safety are typically cued using simple visual stimuli similar to those used in Experiments 1 and 2. In comparison with perception of natural scenes, extracting information from these cues is straightforward and should have a more limited representation in memory. As a result the presentation of threat and safety cues likely doesn't involve or necessitate reentrant modulation of the visual system. Consistent with this view, signal change during threat and safe periods did not differ in secondary visual or dorsal parietal areas in Experiment 2.

Brain Regions Mediating Aversion to Threat of Shock

The affective component of threat of shock is best defined as a region or network of regions that are also activated in the context of aversive perception. This is based on evidence that the profile of defensive responses observed in Experiment 1 resembles similar physiological changes prompted during unpleasant picture processing (e.g. Bradley et al., 2001). Threat of shock and aversive perception are therefore hypothesized to have a common motivational foundation and activate in part similar motivational structures and circuits (Bradley et al., 2005). Experiment 2 demonstrated this to be the case, as several regions activated during threat of shock were also activated when participants viewed unpleasant pictures.

Interestingly, there was no cumulative effect on signal change in these regions when unpleasant pictures were viewed under threat of shock. Instead activation of these regions was equivalent whether unpleasant pictures were viewed under safety or threat of shock. Equivalent responses were also elicited by threat cues presented without a picture and when neutral pictures were viewed during threat periods. Thus, these regions showed heightened activation whenever an aversive stimulus was present.

While this network contained six regions, the anterior insula and the superior medial frontal gyrus are most likely to be involved in mediating the aversiveness of threat of shock. This is based on the neuroanatomical connectivity of these two regions with other structures activated during threat of shock, with subcortical and brainstem regions controlling the autonomic and somatic reactions demonstrated in Experiment 1, and functional evidence that both regions are activated across a variety of emotional induction contexts (Kober et al., 2008; Craig, 2009). Moreover, the anterior insula and superior medial frontal gyrus are the two regions most reliably activated during threat of shock (Mechias, Etkin, Kalisch, 2010).

There is considerable evidence that the anterior insula may mediate the aversiveness of shock anticipation. In humans, the anterior insula is activated in a wide variety of affective contexts, both unpleasant and pleasant (Craig, 2009; Kober et al., 2008). Exaggerated activation of the anterior insula is also reported during fear conditioning in anxiety disorder patients diagnosed with a specific phobia or social phobia compared to healthy controls (Etkin & Wager, 2007). Both these findings fit well with the finding in Experiment 2 that the anterior insula was activated during both aversive perception and threat of shock and that activation was especially heightened in participants reporting high dental fear. Particularly interesting is a recent report that compared emotional ratings for pleasant, neutral, and unpleasant pictures among healthy controls and patients that had suffered insula or amygdala lesions (Berntson, Norman, Bechara, Bruss, Tranel & Cacioppo, 2011). When patients with insula lesions viewed and rated emotional pictures, they generally rated unpleasant pictures as less unpleasant and arousing than patients with amygdala lesions or healthy controls. This

finding supports the suggestion that the anterior insula contributes to the subjective experience of emotion (Craig, 2009).

Anterior insula activation is also positively correlated with changes in autonomic activity (Crtichley, 2005; Crtichley, Elliot, Mathias & Dolan, 2000) and was previously found to correlate with the magnitude of skin conductance responses during threat of shock (Phelps et al. 2001). These findings are consistent with the known neuroanatomical connections of the anterior insula with other limbic sites, including the amygdala and brainstem regions involved in controlling autonomic and somatic reactions (Flynn, 1999; Augustine, 1996; Mesulam & Mufson, 1985). Particularly interesting is evidence that the anterior insula projects to the nucleus reticularis pontis caudalis (Davis et al., 1982). This projection mirrors that of the central nucleus of the amygdala and supports the conjecture that the anterior insula may be able to directly modulate startle reflex magnitude in the same way that the amygdala does during classical fear conditioning (e.g., Greenwald et al., 2003) and emotional perception (Lang & Davis, 2006; Lang, 1995).

Recent studies that compared the neuroanatomy of the anterior insula across species noted a sizeable expansion of this structure in humans, relative to non-human primates (Allman, Tetreault, Hakeem, Manaye, Semendeferi, Erwin, Park, Goubert, & Hof, 2010; Butti & Hof, 2010; Craig, 2009). Interestingly, this expansion seems to be due in part an increase in the number of von Economo neurons (Allman et al., 2010), large bipolar neurons found in the anterior insula and cingulate gyrus in humans and non-human primates. These cells have features characteristic of cortical projection neurons and their distribution in frontoinsular cortex appears to reflect the organization

of the autonomic nervous system (Allman et al., 2010). It is possible that this expansion allows instructed fear to rely more on the anterior insula than the amygdala, and because projections of the anterior insula mirror those of the amygdala, threat of shock and aversive perception elicit similar changes in reflex physiology. If the anterior insula is responsible for startle potentiation in the context of threat of shock—via projections to the PnC—this might also explain why there is no additional increase in startle reflex potentiation when unpleasant pictures themselves are used to cue threat of shock, compared to when pictures cue safety (Bradley, Moulder & Lang, 2005). Converging inputs from the amygdala—reflecting emotional discrimination of picture content—and anterior insula might interact within the acoustic startle pathway, particularly in the PnC (Walker & Davis, 2002). If this is the case it will be interesting in future studies to integrate threat of shock and picture viewing paradigms in novel ways to explore the neural circuitry underlying fear anxiety, and affective startle modulation.

Similar claims can be made for the possible role of the superior medial frontal gyrus in mediating the aversiveness of threat of shock. Like the anterior insula, it is also consistently activated across neuroimaging studies of emotion (Kober et al., 2008; Phan, Wager, Taylor & Liberzon, 2002) and there is neuroanatomical evidence across species that dorsomedial prefrontal cortex projects to limbic and subcortical circuits involved in mediating motor and autonomic output (Ongur & Price, 2000). There is also functional evidence that during emotional processing increased activation of the superior medial frontal gyrus coincides with increased activation of the hypothalamus and periaqueductal gray (PAG), as well as with the amygdala (Kober et al., 2008). In rodents, high levels of dorsal PAG activity is known to inhibit PnC activity and dampen

fear-potentiated startle, mediated by the amygdala. Reflex antagonism might also explain why startle is not further potentiated when unpleasant pictures cue threat of shock (Bradley et al., 2005). That is, if viewing unpleasant pictures under threat of shock sufficiently heightens superior medial frontal gyrus activation to increase dorsal PAG activity, in parallel with increased amygdala activation.

In addition, recent studies have indicated that in rodents, the prelimbic cortex—a likely homologue of the medial frontal gyrus in humans—encodes and retains fear associations to mediate fear expression (for review see Sotres-Bayon & Quirk, 2010; Knapska & Maren, 2009; Burgos-Robles, Vidal-Gonzalez & Quirk, 2009; Laviolette, Lipski & Grace, 2005). Prelimbic mediated excitation of fear expression is also hypothesized to account for the persistence of fear-potentiated startle in rhesus monkeys even after lesioning of the amygdala (Antoniadis, Winslow, Davis & Amaral, 2009; Davis et al., 2008). Interestingly, during fear conditioning prelimbic neurons show sustained increases in activity that last up to several seconds, and match the duration of cue-elicited fear behaviors (e.g., freezing; Burgos-Robles, Vidal-Gonzalez & Quirk, 2009). Activation of the superior medial frontal gyrus is also correlated with the amplitude of the contingent negative variation, a slow, negative cortical potential lasting several seconds that occurs in anticipation of a forthcoming event—especially if that event is motivationally significant (Khader, Schicke, Roder & Rosler, 2008; Nagai, Critchley, Featherstone, Fenwick, Trimble & Dolan, 2004). While the neurophysiological basis of the BOLD signal remains under investigation—there is evidence that it is related to both increases in neural spiking (e.g., Lee, Durand, Gradinaru, Zhang, Goshen, Kim, Fenno, Ramakrishnan & Deisseroth, 2010) and or changes in local field

potentials (Logothetis, 2008; Goense & Logothetis, 2008)—sustained changes in neuronal activity should lead to pronounced increases in BOLD signal change. The finding in Experiment 2 that signal increases in the superior medial frontal gyrus were sustained for the entire threat period (see the upper left hand panel of Figure 3-5 B) is consistent with cross-species evidence that activity in medial prefrontal cortex mediates the expression of anticipatory fear responses.

Brain Regions Involved in Instructed Fear Reversal

Given the speed with which instructions reversed defensive responding in Experiment 1 (e.g., within one trial), regions activated during threat of shock in Experiment 2 are predicted to similarly show a rapid change in activation when initial cue associations with threat and safety are reversed. One possibility is that being instructed about the new contingencies allows one or more of the regions already activated to update the cue associations previously encoded in this network. Consistent with this idea both the anterior insula and superior medial frontal gyrus have been implicated in reversal learning and attention shifting tasks (Wager, Jonides & Reading, 2004; Wager & Barrett, 2004). It is possible then that as part of a defensive network these regions have a specific, integral role in the rapid and flexible identification of environmental threats. In this way their function might parallel that of the amygdala, and in fact, it was recently proposed that the anterior insula anchors a salience detection network that functions to appropriately guide behavior and cognition (Menon & Uddin, 2010).

Another possibility is that the instructed reversal of threat contingencies will activate additional brain regions previously shown to be involved in the reversal of Pavlovian conditioned fear. Two previous functional imaging studies have examined the

neural processes underlying reversal of Pavlovian conditioned fear in the human brain (Schiller, Levy, Niv, LeDoux & Phelps, 2008; Morris & Dolan, 2004). Schiller and colleagues (2008) found that when reinforcement contingencies were reversed brain regions activated during fear acquisition, including the amygdala and anterior insula, continued to respond based on the initial contingencies until halfway through the reversal phase. After this point activation patterns shifted to signal that a non-reinforced cue now predicted aversive reinforcement. Morris and Dolan (2004) similarly found perseverative activation in the amygdala and anterior insula, although it lasted through the reversal phase.

In parallel with delayed or preseverative brain activation during fear reversal, changes in activation of ventromedial prefrontal cortex (vmPFC; Schiller et al., 2008) were shown to flexibly track reversal of the shock contingencies. Previous studies have reported signal decreases in vmPFC during fear acquisition and extinction (e.g., Phelps, Delgado, Nearing & Ledoux, 2004). Schiller and colleagues (2008) similarly found that cues paired with shock elicited signal decreases in vmPFC. Following a reversal of the reinforcement contingencies, the same cues now elicited signal increases in vmPFC, suggesting that they were processed as safety signals.

Fear-related signal decreases in vmPFC found during acquisition and reversal of Pavlovian conditioned fear (e.g., Phelps et al, 2004; Schiller et al., 2008) are not reported to occur during threat of shock, and were not found in Experiment 2. Converging evidence from animal and human studies suggests vmPFC inhibits amygdala output to facilitate fear extinction (Phelps et al., 2004; Quirk & Mueller, 2008). Consistent with these findings, fear reversal of vmPFC activation preceded reversal of

the fear-related amygdala activation (Schiller et al., 2008). Considering meta-analytic evidence that the amygdala is not reliably activated during threat of shock (Mechias, Etkin & Kallisch, 2010) this might explain why deactivation of vmPFC is not found during threat of shock and casts doubt on its possible involvement in the instructed reversal of fear.

A region more likely involved in the instructed reversal of fear is lateral orbitofrontal cortex (lOFC). Threat of shock reliably elicits signal increases in the ventral aspect of the inferior frontal gyrus—inferior and anterior to the horizontal ramus—and reported activations often extend into lOFC (Drabant, et al, 2011; Schunck et al., 2008; Kumari et al., 2007; Dalton et al., 2005). This was the case in Experiment 2 as the activated cluster in the ventral aspect of the inferior frontal gyrus extended into the caudal portion of the lateral orbital gyrus (this occurred bilaterally but was somewhat larger in extent in the right hemisphere). This is consistent with electrophysiology studies in rodents that demonstrated anticipatory firing of orbitofrontal cortex neurons in anticipation of the expected outcome of appetitive and aversive cues (Schoenbaum, Chiba & Gallagher, 1998; Schoenbaum, Setlow, Saddoris & Gallagher, 2003; Takahashi, Roesch, Stalnaker, Haney, Calu, Taylor, Burke, Schoenbaum, 2009; Schoenbaum, Roesch, Stalnaker & Takahashi, 2009).

Increased activation in lOFC is also reported in human neuroimaging studies that examined the reversal of instrumental responses in response to reward and punishment. Morris and Dolan (2004) found that during both fear acquisition and following a reversal of the reinforcement contingencies, cues that currently predicted a loud, aversive noise elicited increased activation in lOFC compared to cues unpaired

with noise. Lateral OFC is known to facilitate changes in associative encoding in other brain regions during fear extinction and affective reversal learning (Herry, Ciocchi, Senn, Demmou, Muller & Luthi, 2008; Quirk & Mueller, 2008; Schoenbaum et al., 2009; Saddoris, Gallagher & Schoenbaum, 2006; Hornak, O'Doherty, Bramham, Rolls, Morris, Bullock & Polkey, 2004).

Signal increases in lOFC are specifically found in response to punishment (e.g., loss of money; Elliot, Agnew & Deakin, 2010; O'Doherty, Critchley, Deichmann & Dolan, 2003; O'Doherty, Kringelbach, Rolls, Hornak & Andrews, 2001) and feedback cues signaling a shift in behavior (Elliot et al., 2010; Remijnse, Nielen, Uylings & Veltman, 2005; Kringelbach & Rolls, 2003; O'Doherty et al., 2003). When integrated with evidence that lOFC is also activated during threat of shock, these findings suggest that the lOFC might play a role in instructed reversal of fear. Considering the recent view that orbitofrontal cortex signals expected outcomes in order to guide learning in other brain regions (Schoenbaum et al., 2009), changes in lOFC activation when threat and safe cues reverse in meaning may even account for the rapid and complete reversal of defensive reactions found in the present study. Supporting this view, activation of lateral orbitofrontal cortex is enhanced when participants first learn to successfully reverse their behavior (Ghahremani et al., 2010).

Clinical Implications of Instructed Fear Reversal

The current finding that verbal instructions are effective in both potentiating and attenuating fear reactions suggests that the instructed reversal of fear paradigm is a useful complement to existing conditioning procedures used to study fear and its extinction. It might be especially relevant for understanding how fear and anxiety interferes with the ability to inhibit fear. Because threat of shock relies on an abstract,

cognitive representation of an aversive stimulus, the current design is especially salient for probing mechanisms of fear inhibition in disorders that involve catastrophizing and worry (e.g., panic disorder or generalized anxiety disorder). This view is supported by accumulating evidence that anxiety disorder patients show resistance to extinction (Lissek, Powers, McClure, Phelps, Woldehawariat, Grillon & Pine, 2005) and that panic disorder patients show overgeneralization of conditioned fear (Lissek, Rabin, Heller, Lukenbaugh, Geraci & Pine, 2010). Moreover, studying instructed fear reversal may help in in understanding how instructions reconfigure perceptual and response biases to lessen fear, processes which form the foundation of empirically-supported cognitive-behavioral therapies.

Instructed Fear Reversal and Cognitive Emotion Regulation

The use of instructions to reverse threat and safety contingencies and alter fear reactions parallels studies that have examined how reinterpreting the significance of a stimulus can modify elicited emotional responses (Ochsner & Gross, 2008). Instructed reversal of fear is similar to emotion regulation in the sense that each paradigm explicitly attempts to attenuate experienced emotion by changing the meaning of a stimulus. As found in the present study, successful use of emotion regulation strategies can enhance or attenuate psychophysiological responses to unpleasant pictures, including startle reflex magnitude, skin conductance, and corrugator activity (e.g., Ray, McRae, Ochsner & Gross, 2010; Lee, Shackman, Jackson & Davidson, 2009; Lissek et al.,, 2007; Jackson, Malmstadt, Larson & Davidson, 2000; Gross, 1998).

One advantage of the instructed reversal paradigm is that the change in meaning of a stimulus is directly linked to an experimental manipulation of its features (i.e., changes in reactivity are under stimulus control) whereas the cognitive regulation of

emotion can involve any number of uncontrolled mental strategies for its implementation (e.g., emotional imagery, distraction, distancing, personalization; Jackson et al., 2000; Lissek et al., 2007; McRae, Hughes, Chopra, Gabrieli, Gross & Ochsner, 2010). It has recently been noted that a neuroscientific understanding of conditioned fear can be useful in deriving a more mechanistic account of cognitive emotion regulation (Schiller & Delgado, 2010; Hartley & Phelps, 2010), and recent studies have found overlap in the neural circuitry mediating the extinction, reversal, and cognitive regulation of fear (Delgado, Nearing, LeDoux & Phelps, 2008; Schiller & Delgado, 2010). Because the paradigm introduced here uses language to associate a cue with a cognitive representation of fear, it could provide further insights into how neural mechanisms supporting fear extinction and reversal enable the cognitive regulation of fear.

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BIOGRAPHICAL SKETCH

Vincent Costa is originally from Long Island, NY. He earned his Bachelor of Science in psychology with a minor in neuroscience from Syracuse University in May 2004. He received a Master of Science in behavioral neuroscience in August 2008, and his doctorate in cognitive and behavioral neuroscience in August 2011 from the Department of Psychology at University of Florida.

Vincent's first experience in biological experimentation foreshadowed his later interest in psychophysiology. As a student in Dr. Susan Pitscitello-Pall's junior high school biology class, he monitored changes in the heart rate of daphnia as they swam in different concentrations of glucose and glycerol solution using a light microscope. Although he had intended to pursue a career in the visual arts—Vincent majored in painting, illustration, and sculpture during the first three years he attended Syracuse University—a steady diet of courses in neuroscience and psychology redirected his humanistic interest in the mind, towards detailed questions about how emotion is instantiated in the brain. These interests motivated Vincent to pursue a senior thesis project under the tutelage of Drs. Joshua M. Smyth and Randall S. Jorgensen. His thesis examined how instructions to suppress emotion altered behavioral and autonomic reactions to emotional pictures.

Eager to gain additional training in psychophysiology and neuroimaging—particularly the use of functional magnetic resonance imaging (fMRI)—Vincent moved to Gainesville, Florida in the summer of 2004 to participate in the post-baccalaureate training program directed by Drs. Bradley and Lang at the Center for the Study of Emotion and Attention. He matriculated into the graduate program of the Department of Psychology at the University of Florida in August 2005. For the next six years, under the

continued mentorship of Drs. Bradley and Lang, and most recently Andreas Keil, Vincent combined functional magnetic resonance imaging with psychophysiology to investigate how the brain functions when people imagine, perceive, or fear emotional stimuli. His research was and continues to be multidisciplinary, drawing on methods derived from psychology, neuroscience, signal processing, and engineering. Vincent's graduate research benefitted from the support provided by a National Research Service Award (NRSA) predoctoral fellowship awarded to him by the National Institute of Mental Health. In addition, his research has been recognized with awards from the American Psychosomatic Society, Society for Psychophysiology, and the Albert Bandura Graduate Research Award from the Association for Psychological Science. After completing his doctorate Vincent moved to Washington, D.C. to work with Dr. Bruno Averbeck as postdoctoral fellow in the Laboratory of Neuropsychology at the National Institute of Mental Health.