Stony Brook University

Generalized Anxiety Disorder:

Examination of Fear Generalization and Anticipation of Affective Stimuli

A Dissertation Presented

by

Tsafrir Greenberg

to

The Graduate School

in Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

in

Biopsychology

Stony Brook University

December 2012

Stony Brook University

The Graduate School

Tsafrir Greenberg

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree, hereby recommend

acceptance of this dissertation

Lilianne R. Mujica-Parodi, Ph.D., Dissertation Advisor
Associate Professor, Biomedical Engineering

Turhan Canli, Ph.D., Chairperson of Defense
Associate Professor, Psychology

Brenda Anderson, Ph.D., Associate Professor, Psychology

Greg Hajcak, Ph.D., Associate Professor, Psychology

Tor Wager, Ph.D., Associate Professor, Psychology
and Neuroscience, University of Colorado at Boulder

This dissertation is accepted by the Graduate School

Charles Taber
Interim Dean of the Graduate School

Abstract of the Dissertation

Generalized Anxiety Disorder:

Examination of Fear Generalization and Anticipation of Affective Stimuli

by

Tsafrir Greenberg

Doctor of Philosophy

in

Biopsychology

Stony Brook University

2012

Despite increasing interest in generalized anxiety disorder (GAD) it remains one of the least studied anxiety disorders and little is known about its neurobiology. The aim of the current research study was to investigate the neural basis of GAD from two perspectives: *fear generalization* and *anticipation of affective outcomes*. Thirty-two women with GAD (17 with and 15 without comorbid major depression disorder) and 25 healthy women underwent functional magnetic resonance imaging (fMRI) while completing two tasks: a fear generalization task in which participants were presented with a conditioned stimulus (CS) that co-terminates with a brief electric shock, and generalization stimuli (GS) that range in perceptual similarity to the CS, and an anticipation task in which predetermined cues warned participants of upcoming aversive, positive, 'uncertain' (either aversive or positive) or neutral movie clips. For the generalization task, neural reactivity in the insula, anterior cingulate cortex, supplementary motor

area and caudate followed a generalization gradient, with a peak response to the CS that declines with greater perceptual dissimilarity of the GS to the CS; consistent with participants' self-report and autonomic responses for each stimulus. In contrast, reactivity in the ventromedial prefrontal cortex (vmPFC), a region associated with fear inhibition, showed an opposite response pattern (i.e., largest response to the GS most dissimilar to the CS). Patients with GAD, compared to healthy individuals, exhibited a flatter vmPFC gradient suggestive of deficient recruitment of vmPFC during fear inhibition. Anticipation of all affective clips engaged a common set of regions – the insula, dorsal anterior cingulate cortex, thalamus, caudate, prefrontal cortex and inferior parietal area – involved in various preparatory processes such as sustained attention, increased arousal, appraisal, and regulation. The nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC), regions implicated in reward processing, were selectively engaged during anticipation of positive clips and the mid-insula was selectively engaged during aversive anticipation. Anticipation during the 'uncertain' condition reflected a preparatory response for both aversive and positive stimuli showing activation in the NAcc, mPFC and mid-insula. Patients with GAD, compared to healthy individuals, exhibited enhanced reactivity during anticipation of neutral clips and a selective increase in visual cortical activation during affective anticipation consistent with a less discriminant anticipatory response. Findings underscore two processes – deficits in fear inhibition of stimuli that resemble conditioned danger cues and excessive anticipation of innocuous stimuli – which may contribute to symptoms of worry and anxiety in GAD.

Dedication

To my parents……באהבה

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACC	anterior cingulate cortex
aINS	anterior insula
ANOVA	analysis of variance
ASI	anxiety sensitivity index
BA	Brodmann area
BDI	Beck depression inventory
BDNF	brain-derived neurotrophic factor
BOLD	blood oxygen level-dependent
CBT	cognitive behavioral therapy
CS	conditioned stimulus
dACC	dorsal anterior cingulate cortex
dlPFC	dorsolateral prefrontal cortex
DPSS-R	disgust propensity and sensitivity scale-revised
DSM	diagnostic and statistical manual of mental disorders
EPI	echo planar imaging
fMRI	functional magnetic resonance imaging
FOV	field of view
FWE	family-wise error
GAD	generalized anxiety disorder
GS	generalization stimuli
IAPS	the international affective picture system
IPA	inferior parietal area
IUS	intolerance of uncertainty scale
lPFC	lateral prefrontal cortex
MASQ	mood and anxiety symptom questionnaire
MASQ-AA	mood and anxiety symptom questionnaire - anxious arousal subscale
MASQ-AD	mood and anxiety symptom questionnaire - anhedonic depression subscale
MASQ-GDA	mood and anxiety symptom questionnaire - general distress anxiety subscale
MASQ-GDD	mood and anxiety symptom questionnaire - general distress depression subscale

MDD	major depression disorder
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NAcc	nucleus accumbens
PE	prediction error
PFC	prefrontal cortex
pINS	posterior insula
PPI	psycho-physiological interaction
PSWQ	Penn state worry questionnaire
PTSD	post-traumatic stress disorder
ROI	region of interest
SAD	social anxiety disorder
SCID	structured clinical interview for the diagnostic and statistical manual of mental disorders
SD	standard deviation
SMA	supplementary motor area
SPM	statistical parametric mapping
STAI-T	trait version of the state-trait anxiety inventory
SVC	small volume correction
TE	echo time
TR	repetition time
UCS	unconditioned stimulus
vmPFC	ventromedial prefrontal cortex

Acknowledgments

Chapter 4 of this dissertation is a reprint of the manuscript titled '*Neural reactivity tracks fear generalization gradients*' as it appears in the journal of Biological Psychology (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2011). Chapter 5 is a reprint of the manuscript titled '*Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization*' as it appears in Depression and Anxiety (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2012).

Chapter 1: General Introduction

Generalized anxiety disorder (GAD) is characterized by excessive and uncontrollable worry about a number of events or activities, accompanied by at least three of the following symptoms: restlessness, fatigue, difficulty concentrating, irritability, muscle tension and difficulty sleeping (American Psychiatric Association, 1994). Estimates for lifetime prevalence of GAD range from 1.9% to 5.4% (Brown, O'Leary, & Barlow, 2001) with women twice as likely to be diagnosed with the disorder (Wittchen, Zhao, Kessler, & Eaton, 1994). GAD is often comorbid with other anxiety or mood disorders with rates ranging from 45% to 98% (Goisman, Goldenberg, Vasile, & Keller, 1995). Comorbidity with major depression disorder (MDD) is of particular interest because of high rates (Kessler, Chiu, Demler, Merikangas, & Walters, 2005) and some overlap in symptoms between the two disorders.

Starting with the diagnostic and statistical manual of mental disorders, third edition revised (DSM-III-R; American Psychiatric Association, 1987) worry was established as the key diagnostic symptom of GAD. Worry is a verbal-semantic process involving uncontrollable thinking about the possibility of future negative events. In the short run, worry reduces threatening imagery and somatic arousal but overtime may interfere with emotional processing and help maintain anxiety-producing cognitions (Borkovec, 1994). The content of worry in GAD is similar to that of other psychiatric disorders and healthy individuals (Abel & Borkovec, 1995). However, GAD patients worry with greater frequency, typically have worries in more than one domain and report more difficulties controlling their worry behavior (Borkovec, 1994; Borkovec, Shadick, & Hopkins, 1991).

Although GAD is associated with substantial human (encompassing social, family and occupational functioning as well as perceived emotional and physical well-being) and economic

burden (Hoffman, Dukes, & Wittchen, 2008) it remains one of the least studied anxiety disorders (Dugas, Anderson, Deschenes, & Donegan, 2010). A common explanation for this paucity of research is that it has taken a long time for the diagnosis of GAD to establish validity, given its early residual status and substantial revisions in diagnostic criteria over the years (Mennin, Heimberg, & Turk, 2004). Investigation of the neurobiology of GAD has lagged behind other anxiety disorders in particular. Findings from the few available neuroimaging studies have been mixed regarding the brain regions underlying its psychopathology (Shin & Liberzon, 2010). Enhanced amygdala activation in GAD has been observed during aversive anticipation (Nitschke et al., 2009) and in response to fearful (E. B. McClure, Monk, et al., 2007) and masked angry faces (Monk et al., 2008) but not in two other studies (Blair et al., 2008; Whalen et al., 2008). Furthermore, contrasting results have been reported with respect to prediction of treatment outcome based on pre-treatment levels of amygdala response (E. B. McClure, Adler, et al., 2007; Whalen et al., 2008). The role of the frontal cortex in GAD requires clarification as well. Enhanced activation in dorsal and rostral ACC has been reported in response to fearful faces (E. B. McClure, Monk, et al., 2007) and in rostral ACC during a decision-making task (Krain et al., 2008). In contrast, patients exhibited a diminished response in pregenual ACC during implicit regulation of emotional conflict (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009).

The aim of the present research was to investigate the neural basis of GAD from two perspectives: *fear generalization* and *anticipation of affective outcomes*.

1.1. *Fear generalization*: Fear learning mechanisms such as fear conditioning and extinction are central to etiological accounts and treatment of pathological anxiety (Mineka & Oehlberg, 2008). Paradigms designed to test these mechanisms have been developed and studied extensively in

animals and adapted for human studies (Delgado, Olsson, & Phelps, 2006). A related learning mechanism that received less attention in humans is fear generalization, which describes the transfer of a conditioned fear response to new stimuli that are similar to the conditioned stimulus (CS). Fear generalization has gained interest in recent years due to its hypothesized relevance to the transfer of fear responses from threat-related stimuli to potentially innocuous cues – a process that appears to be prevalent in most anxiety disorders. Aside from clinical observation, this hypothesis was motivated by evidence that anxiety patients, compared to healthy individuals, show enhanced fear-responding to safety cues during fear-conditioning (Lissek et al., 2005).

Recent studies have validated laboratory-based procedures for testing fear generalization using fear-potentiated startle (Hajcak et al., 2009; Lissek et al., 2008) and skin conductance (Dunsmoor, Mitroff, & LaBar, 2009; Vervliet, Kindt, Vansteenwegen, & Hermans, 2010) to quantify fear responses to a CS and to generalization stimuli (GS) that vary in perceptual similarity to the CS. Such paradigms generate generalization gradients with a peak response to the CS that declines with increasing dissimilarity of the GS to the CS. In healthy individuals, this generalization gradient is characterized by a steep linear slope with sharp decreases across consecutive GS. In patients with panic disorder, on the other hand, the generalization gradient has a flatter linear slope with gradual decreases across stimuli, reflecting greater generalization (Lissek et al., 2010). Overgeneralization of the fear response to stimuli that resemble an initial danger cue may help maintain symptoms by increasing the number of cues in the environment that can trigger additional panic attacks. Such cues can be external and/or internal (e.g., autonomic changes) underscoring the range of stimuli that are potentially involved in the generalization process. In GAD, a similar process of overgeneralization may contribute to an increase in the number of cues/events that elicit worry behavior. This prediction of

overgeneralization in GAD is supported by findings of increased sensitivity to uncertainty (Dugas, Marchand, & Ladouceur, 2005) and a proclivity to interpret neutral/ambiguous stimuli as threatening (Eysenck, Mogg, May, Richards, & Mathews, 1991; Mathews, Richards, & Eysenck, 1989) among GAD patients (Lissek, 2012).

The neurocircuitry supporting fear generalization is unknown. Of relevance, however, are brain regions involved in acquisition and inhibition of fear responses identified with paradigms of fear conditioning and extinction. In human neuroimaging studies, the amygdala, ACC and insula are most consistently activated during fear acquisition showing stronger responses to a conditioned stimulus compared to an unconditioned stimulus (e.g., Büchel, Morris, Dolan, & Friston, 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Morris & Dolan, 2004). Additional regions involved in acquisition and expression of fear are the thalamus, striatum, hippocampus, premotor areas and stimulus-relevant sensory areas (Sehlmeyer et al., 2009). During extinction, activation in the ventromedial prefrontal cortex (vmPFC) has been observed (e.g., Gottfried & Dolan, 2004) consistent with its role in extinction recall and fear inhibition. The interaction between the vmPFC and hippocampus is of potential importance for fear generalization. Both regions are jointly activated in response to an extinguished versus unextinguished CS during the extinction phase (Milad et al., 2007). The hippocampus is involved in contextual modulation of fear expression (Corcoran & Quirk, 2007) and is thought to mediate stimulus representation (Gluck & Myers, 1993). Notably, hippocampally lesioned rats show increased generalization (Wild & Blampied, 1972). In the context of fear generalization, the hippocampus may affect vmPFC modulation of the fear response such that reactivity to the CS is enhanced and reactivity to the GS is inhibited.

For the current research study we optimized a previously validated generalization paradigm (Hajcak et al., 2009) for functional imaging. The aims were: (1) to identify brain regions involved in fear generalization; (2) to determine whether neural reactivity in these regions shows a similar generalization gradient to that reported with other psychophysiological measures of fear (i.e., fear-potentiated startle and skin conductance), and (3) to test whether the slope of such neural gradients, indexing generalization, differs in GAD and healthy individuals. In addition, we examined connectivity of primary regions engaged by the generalization task using psychophysiological interaction analyses. Regions of interest were informed by previous studies of fear conditioning and extinction (Gottfried & Dolan, 2004; LaBar et al., 1998; Sehlmeyer et al., 2009) and included the amygdala, insula, thalamus, caudate, ACC and vmPFC. We hypothesized that reactivity in one or more of these regions would demonstrate a similar gradient response to the pattern reported in laboratory-based studies. Based on evidence for overgeneralization in anxious individuals (Dunsmoor, White, & LaBar, 2011; Lissek et al., 2008), we predicted that patients with GAD would demonstrate greater generalization as evidenced by flatter neural gradient slopes compared to healthy individuals.

1.2. *Anticipation*: Exaggerated anticipation is a central feature of anxiety disorders. Besides feeling anxious in the presence of certain stimuli/events, individuals with anxiety disorders often experience severe anxiety in expectation of confronting these stimuli/events (Barlow, 2000). Importantly, anticipatory anxiety leads to avoidance behavior that could further contribute to the maintenance of symptoms by interfering with extinction. Most neuroimaging studies of anxiety examined brain activation during stimuli presentation – findings showing differential reactivity between anxious individuals and healthy comparisons to disorder-specific and generally aversive

stimuli (e.g., Etkin & Wager, 2007). In addition, there is accumulating evidence for group differences in brain reactivity during the anticipatory phase.

Anticipatory anxiety has been typically assessed for brief periods preceding aversive images (Nitschke, Sarinopoulos, Mackiewicz, Schaefer, & Davidson, 2006; Simmons, Matthews, Stein, & Paulus, 2004; Simmons, Strigo, Matthews, Paulus, & Stein, 2006). Such paradigms most consistently engage the insula. Other regions implicated are the ACC, amygdala and prefrontal cortex. Anxiety-prone individuals (defined on the basis of high trait anxiety) show greater insula activation during aversive anticipation, relative to healthy comparisons (Simmons et al., 2006). In GAD, enhanced amygdala activation has been observed during anticipation of both aversive and neutral pictures, suggesting that patient's anticipatory response is less discriminate and triggered by a broader range of stimuli (Nitschke et al., 2009).

For the current study we used a modified anticipation task designed to better characterize the anticipatory response and facilitate investigation of potential differences in this response between GAD patients and healthy individuals. The task included multiple conditions of anticipation: *aversive anticipation, positive anticipation, 'uncertain' anticipation* and *neutral anticipation*. Target stimuli were short movie clips depicting a medical procedure (aversive condition), sexual situations (positive condition) and a person driving (neutral condition). For the 'uncertain' condition, either positive or aversive clips were presented with unknown probability. We used longer anticipation periods (16 seconds), compared to previous studies, to test whether the same regions are engaged when anticipated events are more distal and to examine the temporal dynamics of the anticipatory response. We also collected self-report measures at the end of each trial to gauge participants' subjective experience of anticipatory anxiety and its association with neural reactivity, as well as autonomic measures of arousal

during the anticipation phase.

The aims were: (1) to establish normative sustained anticipatory responses for high arousal aversive and positive stimuli and a condition of uncertainty, and (2) to investigate whether these responses deviate in patients with GAD. We hypothesized that anticipation of aversive, positive and 'uncertain' clips would recruit common regions previously reported in studies of aversive anticipation (Nitschke et al., 2006) and that additional regions associated with appetitive processing such as the nucleus accumbens (NAcc) and mPFC would be preferentially engaged during anticipation of positive clips. The insula, which has been implicated in processing disgust, was expected to be preferentially engaged during aversive anticipation. Based on previous findings demonstrating an enhanced anticipatory response in anxious individuals (Simmons et al., 2011) and heightened amygdala activation in GAD during anticipation of both aversive and neutral images(Nitschke et al., 2009) , we hypothesized that GAD patients would exhibit greater anticipatory reactivity and reduced discriminability across conditions compared to healthy individuals. Finally, based on evidence for greater intolerance of uncertainty (Ladouceur et al., 1999) and a tendency to interpret ambiguous/neutral stimuli as threatening (Eysenck et al., 1991; Mathews et al., 1989) among GAD patients, we expected greater disparity in anticipatory reactivity between groups for the 'uncertain' and neutral conditions.

1.3 Overview of chapters

In the current study we investigated the neural correlates of fear generalization and anticipation in a group of women diagnosed with GAD (N=32) and a group of healthy women (N=25). Chapter 2 presents the methods for the study. Chapter 3 presents a summary of demographics/descriptives and self-report measures for all participants. Chapter 4 is a reprint of

the manuscript '*Neural reactivity tracks fear generalization gradients'* (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2011) and presents findings for the generalization task in the Healthy group and a pilot sample (N =8). Chapter 5 is a reprint of the manuscript '*Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization*' (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2012) and presents findings for the generalization task in the GAD group and between-group comparisons with the Healthy group. Chapter 6 presents findings for the anticipation task in the Healthy group. Chapter 7 presents findings for the anticipation task in the GAD group and between-group comparisons with the Healthy group. Finally, Chapter 8 presents a general discussion for the entire study.

Chapter 2: General Methods for the Study

2.1 *Participants*

Fifty-seven women participated in the study, 15 with a current DSM-IV diagnosis of GAD (*Mean age* = 22.1; *SD* = 4.3), 17 with a diagnosis of GAD and comorbid major depression disorder (MDD; *Mean age* = 22.4; *SD* = 4.8) and 25 healthy individuals (*Mean age* = 21.6; *SD* = 5.1); ages for the entire sample ranged from 18 to 44. The study included only female participants to reflect biases in diagnoses and research participation for GAD. Participants were recruited from the Stony Brook University campus, Suffolk community, and online websites. The Structured Clinical Interview for DSM-IV Axis I Disorders -Patient Edition, Version 2 (SCID-I/P; First, Spitzer, Gibbon, & Williams, 2002) was administered to confirm diagnoses of GAD in the patient groups and absence of Axis I diagnoses in the Healthy group. None of the participants were currently using any psychotropic medications. Forty-nine participants were right handed, four were left handed and four were ambidextrous. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

2.2 *Study procedure*

Potential participants completed a phone screening to determine whether they met general requirements for the study which included: age between 18-50, no history of psychosis or substance dependence, no intake of any psychiatric medication for at least two months, eligibility for MRI (i.e., no metal in body, not pregnant or lactating, no history of brain injury or neurological disorder, no claustrophobia) and likelihood to fulfill criteria for the GAD, Comorbid, or Healthy groups based on responses to a modified version of the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998) and a semi-structured diagnostic interview designed to screen for 17 Axis I disorders. Those who met these requirements were scheduled for

a SCID interview. The inclusion criteria for the GAD group were: 1) current diagnosis of GAD with no history of MDD in the past 12 months and 2) absence of any other Axis I disorder except for specific phobia. For the Comorbid group they were: 1) current diagnosis of GAD and MDD, and 2) absence of any other Axis I disorder except for specific phobia, and for the Healthy group they were absence of any current or past Axis I diagnosis. Individuals who met criteria for any of the experimental groups were scheduled for an fMRI session at the Social, Cognitive and Affective Neuroscience (SCAN) imaging center at Stony Brook University. The fMRI session consisted of a structural scan and two functional tasks: *generalization* and *anticipation*. We used an eye tracker (Eyelink 1000; SR Research Ltd., Ontario, Canada) to monitor participants' compliance during the tasks. Following the fMRI session, participants completed self-report questionnaires.

2.3 *fMRI tasks*

The generalization and anticipation tasks were programmed with Experiment Builder (SR Research Ltd.) and presented with an MRI compatible 60 Hz projector with 1024 × 768 resolution (Psychology Software Tools, Inc., Sharpsburg, PA). During the tasks we collected pupil response measures (Eyelink 1000; SR Research Ltd.) to assess participants' arousal levels.

2.3.1 Generalization task

2.3.1.1 Procedure

Prior to the scan, an electric shock, delivered to the left wrist (Constant Voltage Stimulator STM 200; Biopac Systems Inc., Goleta, CA), was individually set for each participant to a level that was "uncomfortable but not painful". Shock levels were comparable across experimental groups. Instructions for the task were then provided. Participants were told that the

middle-sized rectangle (CS) indicated a 50% probability that they would receive a subsequent electric shock, but that shocks would never follow rectangles of greater or lesser size. A conditioning phase was administered next, which included five presentations of the CS with electric shock (i.e., CS_{paired}) and one presentation of each of the other six rectangles. The task immediately followed.

2.3.1.2 Task design

The task consisted of three blocks presented consecutively. Each block included 40 trials (5 trials × 8 conditions) for a total of 120 trials. The stimuli were seven red rectangles with identical height (56 pixels) and varying width (112 to 448 pixels) presented against a black background. The middle-sized rectangle (280 pixels) was the conditioned stimulus (CS). Half of the time the CS co-terminated with a 500 ms electric shock (CS_{paired}), while half of the time it did not ($CS_{unpaired}$). The six remaining rectangles differed by ±20%, ±40% or ±60% in width from the CS and served as the generalization stimuli (GS). Stimuli were presented pseudorandomly for 2 seconds with a jittered interstimulus interval ranging from 4 to 10 seconds, during which a white fixation cross was shown on a black background. The duration of the task was 15 minutes and 24 seconds. Following the task, participants rated the likelihood of having been shocked for each rectangle, on a Likert-type scale of 1 (“certainly not shocked”) to 5 (“certainly shocked”).

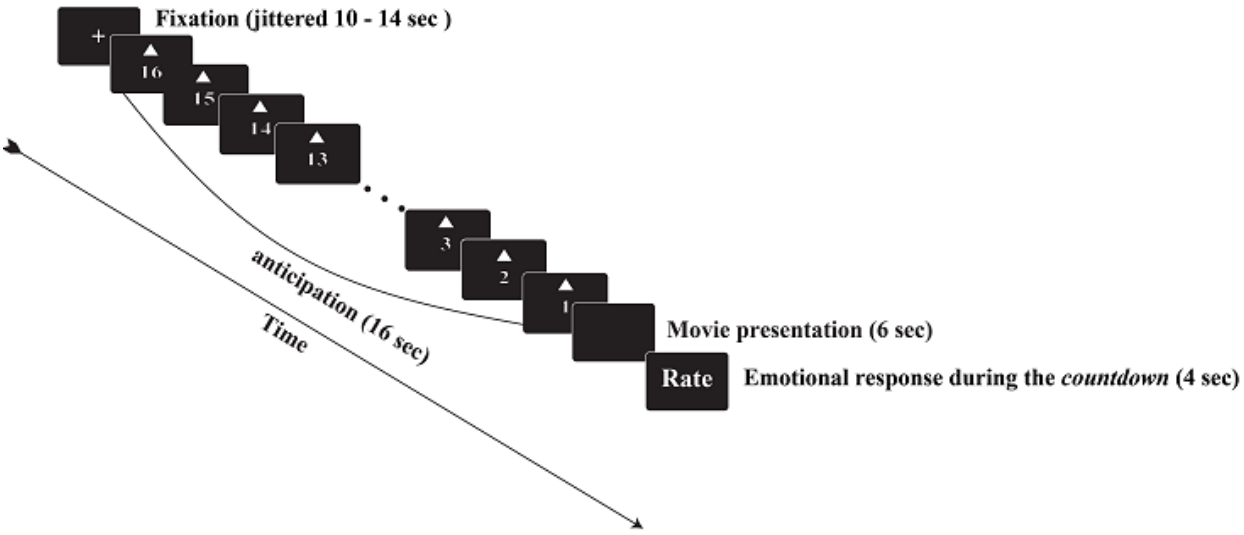
2.3.2 Anticipation task

2.3.2.1 Task design

The Anticipation task consisted of 40 trials presented pseudorandomly: 10 aversive trials (anticipation of an aversive movie clip), 10 positive trials (anticipation of a positive movie clip), 10 neutral trials (anticipation of a neutral movie clip), and 10 ‘uncertain’ trials (anticipation of

either a positive or aversive movie clip; 50% each). As shown in figure 1, each trial began with a white fixation crosshair presented in the center of a black screen (jittered 10 - 14 seconds). The fixation crosshair was followed by an anticipation period during which a countdown from 16 to 1 was numerically presented in the center of the screen with a specific cue associated with each trial type presented above it (16 seconds duration; the cues were: '▼' for an aversive clip, '▲' for a positive clip, '**?**' for an 'uncertain' clip and '**—**' for a neutral clip. A movie clip was presented next (6 seconds) and then participants rated the strength of their emotional response during the countdown period on a 4-point scale (4 seconds). The duration of the task was 25 minutes and 30 seconds.

Figure 1. Illustration of a single trial (anticipation of a positive clip).



2.3.2.2 Stimuli

The aversive clips were taken from a non-commercial surgery film of arm amputation which has been shown to reliably elicit disgust and, to a lesser extent, fear (Gross & Levenson, 1995), and an additional film of a thigh surgery found online. The positive clips were sexually

explicit and taken from two scenes featured in Eyes of Desire 2 (Femme Productions) and Island Fever 2 (Digital Playground), which were pilot rated high on arousal and positive valence by women (N = 8). The neutral clips were taken from the movie *Quiet Earth* (1985) and consisted of a scene showing a man driving.

2.4 *Post-task rating of clips*

After completion of the anticipation task, participants rated each clip for valence and arousal using two 9-point scales. The valence scale ranged from 1 = "negative" to 9 = "positive". The arousal scale ranged from 1 = "calm" to 9 = "excited" (participants were instructed that a high score on this scale could reflect either negative or positive arousal).

2.5 *Image acquisition*

Participants were scanned with a 3 Tesla Siemens Trio scanner at the Social, Cognitive, and Affective Neuroscience center at Stony Brook University. First, we obtained T1-weighted structural scans with repetition time (TR) = 1900 ms, echo time (TE) = 2.53, Flip angle = 9°, field of view (FOV) = 176 × 250 × 250 mm, and Matrix = 176 × 256 × 256 mm. For the Generalization task we acquired a total of 440 T2*-weighted echoplanar images (EPI) with an oblique coronal angle with a TR = 2100 ms, TE = 23 ms, Flip angle = 83°, FOV = 224 × 224 mm, Matrix = 96 × 96 mm, Slices = 37, and slice thickness = 3.5 mm. For the Anticipation task, we acquired 760 T2*-weighted EPI images with an oblique coronal angle with a TR = 2100 ms, TE = 23 ms, Flip angle = 83°, FOV = 224 × 224 mm, Matrix = 96 × 96 mm, Slices = 37, and slice thickness = 3.5 mm.

2.6 *Image analysis*

Preprocessing procedures were performed in SPM8 (www.fil.ion.ucl.ac.uk/spm) and included slice time correction, motion correction, normalization and smoothing with a 6-mm full

width at half maximum Gaussian kernel. Specific procedures for statistical analyses are provided in the relevant chapters.

2.7 *Psychophysiological interaction analysis (PPI)*

PPI is a connectivity technique based on regression models; it identifies which voxels within the entire brain (or within regions of interest) show increased coupling with a seed region, in response to specific conditions of a task (see Friston et al., 1997 for details). The design matrix in PPI includes three variables: 1) A variable that reflects the experimental paradigm. 2) A time-series extracted from the seed region and 3) an interaction variable that represents the interaction between variables 1 and 2. Importantly, the variance associated with the interaction term is not accounted for by the main effects of the task and physiological correlations. Specific procedures for the PPI analysis are provided in the relevant chapters.

2.8 *Pupillary response*

Pupil data was processed using custom MATLAB codes (MathWorks). Specific procedures for preprocessing and analysis are provided in the relevant chapters.

2.9 *Self-report questionnaires*

After the fMRI session participants completed the following self-report questionnaires:

Penn State Worry Questionnaire (PSWQ)

A 16-item scale that assesses the frequency and intensity of worry (Meyer, Miller, Metzger, & Borkovec, 1990) – the primary diagnostic symptom of GAD (American Psychiatric Association, 1994). Each item is rated by participants on a scale of 1 ("not at all typical of me") to 5 ("very typical of me"). The total score of the questionnaire ranges from 16 to 80. The PSWQ has high internal consistency, α ranges from .86 - .95, and good test-retest reliability, r ranges from .74 - .93 (Molina & Borkovec, 1994).

Intolerance of Uncertainty Scale (IUS)

A 27-item scale designed to assess how individuals respond to uncertainty on cognitive, emotional and behavioral levels (Freeston, Rhéaume, Letarte, & Dugas, 1994). Individuals with GAD are thought to be especially sensitive to uncertainty regarding future events which can contribute to their worry behavior (Ladouceur, Gosselin, & Dugas, 2000). Each item is rated on a scale of 1 ("not at all characteristic of me") to 5 ("entirely characteristic of me"). The total score ranges from 27 to 135. The IUS has good test-retest reliability; r = .74 (Dugas, Freeston, & Ladouceur, 1997) and excellent internal consistency; α = .91 (Freeston et al., 1994).

Anxiety Sensitivity Index (ASI)

A 16-item scale designed to assess the extent to which an individual believes that anxiety may lead to harmful physical, psychological, or social consequences (Peterson & Reiss, 1992). Each item is rated on a scale of 0 ("very little") to 4 ("very much"). The total score ranges from 0 to 64. A recent meta-analysis found that of all the anxiety disorders anxiety sensitivity is most strongly associated with panic disorder and GAD (Naragon-Gainey, 2010). The ASI has good internal consistency, α ranging between .82 - .91 and satisfactory test-retest reliability with r ranging from .71 to .75 (Peterson & Reiss, 1993).

Trait version of the State-Trait Anxiety Inventory (STAI-T)

A 20-item scale that measures stable individual differences in proneness to respond with tension, apprehension, and heightened autonomic activity (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Each item is rated on a scale of 1 ("almost never") to 4 ("almost always"). The total score ranges from 20 to 80. The STAI-T has been widely used in both clinical and nonclinical populations. While trait anxiety varies across individuals, those with anxiety disorders reliably exhibit the highest levels (Zinbarg & Barlow, 1996). The STAI-T has high

internal consistency, α ranging between .86-.95 (Spielberger et al., 1983) and high test-retest reliability, average r = .88 (Barnes, Harp, & Jung, 2002).

Beck Depression Inventory Second Edition (BDI-II)

A 21-item self-report measure that assesses the presence and severity of depressive symptoms (Beck, Steer, & Brown, 1996). Participants are asked to choose one of four statements, rated from 0 to 3, which best describes how they have been feeling during the past two weeks. The total score ranges from 0 to 63. A total score of 0 - 13 represents minimal levels of depressive symptoms, 14 - 19 mild levels, 20 - 28 moderate levels, and 29 - 63 severe levels. The scale has high internal consistency, α = .91, and test-retest reliability, r = 0.93 (Beck et al., 1996).

Disgust Propensity and Sensitivity Scale - Revised (DPSS-R)

A 16-item measure designed to assess the extent to which individuals experience disgust (i.e., disgust propensity) and their appraisal of these experiences (i.e., disgust sensitivity; van Overveld, de Jong, Peters, Cavanagh, & Davey, 2006). The two subscales consist of 8 items each. Each item is rated on a scale of 1 ("never") to 5 ("always"). The total score for each subscale ranges from 8 to 40. The DPSS-R has good test-retest reliability with alpha coefficients of 0.78 for the Disgust Propensity subscale and 0.77 for the Disgust Sensitivity subscale (van Overveld et al., 2006). Both disgust propensity and disgust sensitivity can modulate neural responses to aversive/disgusting stimuli (Mataix-Cols et al., 2008) therefore scores from this scale were included as covariates for analysis of the aversive clips presented in the anticipation task.

The Mood and Anxiety Symptom Questionnaire (MASQ)

A 90-item measure that assesses discrete dimensions of anxiety and depression and mixed symptomatology. The scale is based on the tripartite model of affect and was designed to measure its three factors (i.e., anxiety-specific, depression-specific and shared symptoms; Watson & Clark, 1991). The MASQ consists of three general distress subscales: anxious symptoms (11 items), depressive symptoms (12 items) and mixed symptoms (28 items), and two specific subscales: an anxiety-specific subscale – anxious arousal (17 items) – that measures physiological hyperarousal or somatic arousal and a depression-specific subscale – anhedonic depression (22 items) – that measures positive effect and loss of interest/motivation. Participants indicate on a five-point scale, ranging from 1 ("not at all") to 5 ("extremely") to what extent they experienced various symptoms during the past week. Total scores for the various subscales are: 11 to 55 for the general distress anxiety subscale (MASW-GDA), 12 to 60 for the general distress depression subscale (MASQ-GDD), 17 to 85 for the anxious arousal subscale (MASQ-AA) and 22 to 110 for the anhedonic depression subscale (MASQ-AD). Due to high correlations, observed in previous studies (Watson et al., 1995), between the mixed symptoms subscale and both the MASW-GDA and MASQ-GDD, scores for the mixed symptoms subscale were not calculated. MASQ scales have good internal consistency (Watson et al., 1995).

Chapter 3: Summary of Demographics/Descriptives and Self-report Measures

3.1 *Demographics*

The racial representation across study groups was: 26 Caucasians, 14 Asians, 13 African Americans, 3 multiracial (i.e., two or more races) and 1 other. The highest level of education attained by participants was as follows: 42 had one or more years of college, 9 had one or more years of graduate/professional school, 3 attained a Bachelor's degree, 2 attained an Associate's degree, and 1 attained a Master's degree. Chai square tests indicated that the GAD group and Healthy group did not differ on these two variables (both *ps* > .2).

3.2 *Stage of menstrual cycle*

We approximated participants' menstrual phase on the day of testing based on self-report of the first and last date of their last menstrual period prior to the testing session (assuming a 28-day cycle). Based on this information 26 participants were in the Menstrual (1-4 days) or Follicular phase (1-12 days), 5 were in the Ovulation phase (13-15 days) and 22 were in the Luteal phase (16-28 days). In addition, one participant was in the menopause phase and three participants had irregular cycles. A Chai square test for all remaining participants (N= 53) indicated that women in the GAD group and Healthy group did not differ with respect to the phase of their menstrual cycle during testing (p >.5).

3.3 *Self-report questionnaires*

Table 1 shows mean scores and standard deviations for the Penn State Worry Questionnaire (PSWQ), Intolerance of Uncertainty (IUS), trait anxiety scale (STAI-T), Anxiety Sensitivity Index (ASI), Beck Depression Inventory (BDI) and four subscales from the Mood and Anxiety Symptom Questionnaire (the general distress anxiety subscale; MASQ-GD, the general distress depression subscale; MASQ-GDD, the anxious arousal subscale; MASQ-AA

and the anhedonic depression subscale; MASQ-AD) for the GAD-only, Comorbid and Healthy groups. The GAD-only and Comorbid groups scored higher than the Healthy group on all measures consistent with greater severity of symptoms in patients (all *ps* < .001; except for the anxious arousal subscale for which the difference in scores between the Comorbid and Healthy groups was marginally significant $p = .066$). Mean scores for the PSWQ, STAI-T and BDI reflected clinical levels of worry and anxiety (Antony, Orsillo, & Roemer, 2001) and mild to moderate levels of depression in the GAD-only and Comorbid groups. For the Disgust Propensity and Sensitivity Scale-Revised (DPSS-R), scores in the GAD group were higher ($p = .003$) and in the Comorbid group marginally higher ($p = .076$) compared to the Healthy group.

Between-group comparisons for the two patient groups revealed higher scores in the Comorbid group for the MASQ- GDD ($p = .048$) and the MASQ-AD ($p = .003$), and a trend for higher scores on the BDI ($p = .084$), consistent with greater symptoms of depression. All pairwise comparisons were made using the Bonferroni adjustment for multiple comparisons.

3.4 *Correlations between self-report questionnaires*

As shown in Table 2 correlations between the various questionnaires were all significant ($ps \leq .001$). Correlations between the STAI-T, MASQ-GDA and MASQ-AA and between the BDI, MASQ-GDD and MASQ-AD demonstrate good convergent validity for measures of anxiety and depression. However, moderate to high correlations across all questionnaires suggest that in general their discriminant validity is low, particularly for the STAI-T and BDI. The MASQ-AA and MASQ-AD were specifically designed to distinguish anxiety and depressive symptoms and do perform better in that regard. The correlation between the two subscales was the second lowest when examined for the entire sample ($r = .42$; $p = .001$) and higher in the Comorbid group ($r = .47$; $p = .056$) than in the GAD-only group ($r = .33$; $p = .23$).

Table 1. Mean scores and standard deviations for self-report questionnaires for the GAD-only, Comorbid and Healthy groups.

	GAD-only (N=15)		**Comorbid** (N=17)		**Healthy** (N=25)	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
PSWQ	63.67	9.96	65.29	8.21	47.60	10.39
IUS	88.73	17.89	85.12	19.06	52.56	11.47
STAI-T	52.67	10.98	57.24	8.99	37.52	6.52
ASI	34.13	11.85	33.18	13.86	16.72	5.97
BDI (affective)	6.87	4.45	9.29	5.43	1.88	1.86
BDI (somatic)	11.47	9.09	16.59	6.94	2.88	2.49
BDI (total)	18.33	13.07	25.88	11.53	4.76	3.54
MASQ-GDA	25.80	9.64	26.47	5.17	16.80	4.03
MASQ-GDD	30.53	12.63	38.59	11.07	17.88	3.60
MASQ-AA	31.13	10.52	26.41	7.47	21.04	4.08
MASQ-AD	67.07	15.85	82.76	11.14	53.44	11.36

Table 2. Bivariate correlations between self-report questionnaires (N = 57).

	PSWQ	**IUS**	**STAI-T**	**ASI**	**BDI total**	**MASQ GDA**	**MASQ GDD**	**MASQ AA**	**MASQ AD**
PSWQ	--	.799*	.758*	.681*	.622*	.632*	.603*	.412*	.603*
IUS		--	.846*	.766*	.753*	.701*	.676*	.562*	.660*
STAI-T			--	.725*	.864*	.721*	.825*	.543*	.858*
ASI				--	.704*	.683*	.585*	.696*	.500*
BDI total					--	.694*	.832*	.614*	.833*
MASQ GDA						--	.685*	.722*	.603*
MASQ GDD							--	.456*	.779*
MASQ AA								--	.415*
MASQ AD									--

PSWQ = Penn State Worry Questionnaire; **IUS** = intolerance of uncertainty; S**TAI-T** = Trait Anxiety inventory; **ASI** = Anxiety Sensitivity index; **BDI affective** = total affective symptoms from the Beck Depression Inventory; **BDI somatic** = total somatic symptoms from the Beck Depression Inventory; **BDI total** = total score from the Beck Depression Inventory**; MASQ-GDA** = Mood and Anxiety Symptom Questionnaire-General Distress scale Anxiety; **MASQ-GDD** = Mood and Anxiety Symptom Questionnaire- General Distress scale Depression; **MASQ-AA** = Mood and Anxiety Symptom Questionnaire-Anxious Arousal; **MASQ-AD** = Mood and Anxiety Symptom Questionnaire-Anhedonic Depression; ***p* ≤ .001**

Chapter 4: Neural Reactivity Tracks Fear Generalization Gradients

4.1 Introduction

Paradigms that assess fear learning have provided valuable translational tools for understanding the etiology, maintenance and treatment of anxiety disorders (Milad, Rauch, Pitman, & Quirk, 2006; Mineka & Oehlberg, 2008). The acquisition and extinction of conditioned fear responses involve a common neurocircuitry across species that includes the amygdala, insula, anterior cingulate cortex, hippocampus, sensory areas, and ventromedial prefrontal cortex (Büchel & Dolan, 2000; LeDoux, 2000; Phelps, Delgado, Nearing, & LeDoux, 2004). In addition to acquisition and extinction, there is increasing interest in fear generalization, which describes the transfer of a conditioned fear response to stimuli that are perceptually similar to the conditioned stimulus (CS). Insofar as the transfer of fear responses from threat-related stimuli to potentially innocuous cues is a common feature in anxiety disorders (Lissek et al., 2008), fear generalization may be a key learning process in the development and maintenance of pathological anxiety.

Recent studies have validated laboratory-based procedures for testing fear generalization, which involves the assessment of fear responses to a CS and to generalization stimuli (GS) that vary in perceptual similarity to the CS (Hajcak et al., 2009; Lissek et al., 2008). In these paradigms, fear responses were quantified with the fear-potentiated startle reflex, which followed a generalization gradient: the strongest startle reflex was elicited during the CS, with a steep decline corresponding to the relative decrease in similarity of the GS to the CS[1] (Hajcak et al., 2009; Lissek et al., 2008). Lissek and colleagues assessed fear generalization in a paradigm in which participants had to learn which stimulus was the CS and which were the GS. On the other

[1] Comparable results have also been obtained using skin conductance (Dunsmoor et al., 2009; Vervliet et al., 2010), which is a more general measure of arousal that is not specific to defensive motivation.

hand, Hajcak and colleagues found comparable results even when participants were explicitly instructed regarding the identity of the CS and the reinforcement contingencies to the CS and GS. Despite being told explicitly which stimulus was the CS, and never being shocked following a GS, participants in the Hajcak et al. study had larger startle responses and reported greater shock likelihood as GS were more perceptually similar to the CS.

Fear generalization paradigms could be useful for assessing pathological fear and risk for anxious psychopathology. For instance, patients with panic disorder exhibit a flatter fear gradient with more gradual decreases in fear response to the GS (Lissek et al., 2010). Hajcak and colleagues (2009) reported fear generalization deficits in a generalization paradigm as a function of variation in the brain-derived neurotrophic factor (BDNF) genotype, which has been related to both learning and anxiety-related behaviors.

In the current study, we sought to extend this work by examining neural activity using fMRI in a fear generalization paradigm that we previously employed (Hajcak et al., 2009). The aim was to elucidate the brain regions associated with generalization, which have received little attention in the literature, and to examine whether reactivity in these regions exhibit a similar generalization gradient to that reported with peripheral measures of fear. These neural gradients may be useful in identifying deficits in the generalization process and may be relevant to future work on pathological anxiety (e.g., Lissek et al., 2010). In the current study, the CS was a middle-sized rectangle, and the GS were six additional rectangles varying in width from the CS by ±20%, ±40% or ±60%.

In an initial experiment (N = 8), we examined regions of interest (ROIs) based on neuroimaging studies of fear learning that have implicated key areas in the expression and inhibition of autonomic and behavioral fear responses.(Dunsmoor, Prince, Murty, Kragel, &

LaBar, 2011; Sehlmeyer et al., 2009). These ROIs included the *amygdala*, *insula*, *thalamus*, *caudate*, *anterior cingulate cortex (ACC), and ventromedial prefrontal cortex (vmPFC)*. We hypothesized that reactivity in one or more of these regions would demonstrate a similar gradient response to the pattern reported in previous laboratory-based studies. In a second experiment (N = 25), we conducted a whole-brain analysis and obtained additional self-report ratings and physiological measures.

4.2 Method: Experiment 1 (Pilot study)

4.2.1 *Participants*

Eight individuals (6 females and 2 males) participated in the study (*Mean age* = 23.2; *SD* = 4.7). All reported being right handed. Potential participants were screened for prescription and recreational drug usage, as well as neurological and psychological histories. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

4.2.2 *Procedure*

Prior to the scan, an electric shock, delivered to the left wrist (Constant Voltage Stimulator STM 200; Biopac Systems Inc.), was individually set for each participant to a level that was "uncomfortable but not painful". Instructions for the task were then provided. Participants were told that the middle-sized rectangle (CS) indicated a 50% probability that they would receive a subsequent electric shock, but that shocks would never follow rectangles of greater or lesser size. A conditioning phase was administered next, which included five presentations of the CS with electric shock (i.e., CS_{paired}) and one presentation of each of the other six rectangles. The task immediately followed. Thus, the current study examined

generalization within the context of a paradigm that combined instructed and associative fear learning.

4.2.3 *Task*

The task consisted of three blocks presented consecutively. Each block included 40 trials (5 trials × 8 conditions) for a total of 120 trials. The stimuli were seven red rectangles with identical height (56 pixels) and varying width (112 to 448 pixels) presented against a black background. The middle-sized rectangle (280 pixels) was the conditioned stimulus (CS). Half of the time the CS co-terminated with a 500 ms electric shock (CS_{paired}), while half of the time it did not ($CS_{unpaired}$). The six remaining rectangles differed by ±20%, ±40% or ±60% in width from the CS and served as the generalization stimuli (GS). Stimuli were presented pseudorandomly for 2 seconds with a jittered interstimulus interval ranging from 4 to 10 seconds, during which a white fixation cross was shown on a black background. The duration of the task was 15 minutes and 12 seconds.

4.2.4 *Image Acquisition*

Participants were scanned with a 3 Tesla Siemens Trio scanner at the Stony Brook Social, Cognitive, and Affective Neuroscience (SCAN) center. A total of 456 T2*-weighted echoplanar images were acquired with an oblique coronal angle and TR = 2000 ms, TE = 22 ms, Flip Angle = 83º, Matrix = 96 × 96, FOV = 224 × 224 mm, Slices = 36 and Slice Thickness = 3.5 mm. In addition, we obtained T1-weighted structural scans with TR = 1900 ms, TE = 2.53, Flip angle = 9°, FOV = 176 × 250 × 250 mm, and Matrix = 176 × 256 × 256 mm.

4.2.5 *Image Analysis*

Preprocessing procedures were performed in SPM8 and included slice time correction, motion correction, normalization and smoothing with a 6-mm full width at half maximum

Gaussian kernel. Preprocessed images were entered into a general linear model in which each rectangle was modeled as an event with no duration; CS_{paired} and $CS_{unpaired}$ were modeled separately. The six motion parameters estimated during realignment were included as regressors of no interest and serial autocorrelations were modeled using an AR (1) process. First-level single subject statistical parameter maps were created for the 'CS_{paired} - Baseline' (i.e., fixation), '$CS_{unpaired}$ - Baseline' and each of the 'GS - Baseline' contrasts. These contrasts, except for 'CS_{paired} - Baseline', were used in a second-level random effects repeated measures analysis.

4.2.6 *Gradients of neural reactivity*

Individual bilateral masks were created for the amygdala, insula, thalamus, caudate nucleus, anterior cingulate cortex (ACC) and ventromedial prefrontal cortex (vmPFC) using the Masks for Regions of Interest Analysis software (Walter et al., 2003). A region of interest (ROI) analysis for the F-contrast (main group effect) was performed using an initial threshold of $\alpha = .01_{(uncorrected)}$ and extent threshold = 20 contiguous voxels, and a small volume familywise error rate corrected $\alpha = .05$, for each mask.

Neural gradients were generated for the right and left insula (which were the only regions that showed significant activation with these thresholds) by extracting the first eigenvariate (i.e., the principal component) from a 6 mm sphere centered on the local maxima within each region, for each of the '$CS_{unpaired}$ - Baseline' and 'GS - Baseline' contrasts, across all participants. Mean values for $CS_{unpaired}$, as well as GS ±20%, GS ±40% and GS ±60%, were plotted as a four-point gradient.

4.3 Results

4.3.1 *Generalization gradients of neural activation*

Generalization gradients for the right and left insula are shown in Figure 2(c) and Figure 2(b), respectively. Reactivity in the right ($F_{(3,21)} = 18$, $p < .001$) and left ($F_{(3,21)} = 13.3$, $p < .001$) insula varied as a function of stimulus type. For the right insula, pairwise comparisons revealed higher reactivity for the $CS_{unpaired}$ versus GS ±40% ($p = .004$) and GS ±60% ($p = .01$), and for the GS ±20% versus GS ±40% ($p = .02$). A comparison of the GS ±20% to GS ±60% was marginally significant ($p = .053$). For the left insula, reactivity was higher for the $CS_{unpaired}$ versus GS ±40% ($p = .007$) and GS ±60% ($p = .03$), and for the GS ±20% versus both GS ±40% ($p = .03$) and GS ±60% ($p = .04$).

4.4 Method: Experiment 2

4.4.1 *Participants*

Twenty-five women participated in the study (*Mean age* = 21.6; *SD* = 5.1). All reported being right-handed except for one participant, who reported being ambidextrous. Participants were screened for psychiatric illness with the Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Edition, Version 2 (SCID-I/P; First et al., 2002). All other screening procedures were identical to Experiment 1. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

4.4.2 *Experimental paradigm*

The experimental paradigm was identical to Experiment 1 except for the addition of post-task ratings of shock likelihood for each rectangle, obtained on a Likert-type scale of 1 ("certainly not shocked") to 5 ("certainly shocked"), acquisition of pupillary response with the

Eyelink-1000 (SR Research Ltd., Ontario, Canada) as a measure of activation of the sympathetic nervous system, as well as a 12 second increase in task length to accommodate a change in TR and TE due to scanner requirements.

4.4.3 *Image Acquisition and Analysis*

A total of 440 T2*-weighted echoplanar images were acquired with an oblique coronal angle and TR = 2100 ms, TE = 23 ms, flip Angle = 83º, matrix = 96 × 96, FOV = 224 × 224 mm, slices = 37 and slice thickness = 3.5 mm. Parameters for acquisition of structural images, as well as preprocessing procedures and statistical analysis were identical to Experiment 1.

4.4.4 *Gradients of neural reactivity*

Gradients of neural reactivity were generated for all brain regions for which we found significant clusters for the main effect group F-contrast using a whole brain threshold of $\alpha = .001_{\text{uncorrected}}$ and extent threshold of 20 contiguous voxels.

4.4.5 *Preprocessing of pupil data*

Pupil data was processed using custom MATLAB codes (MathWorks). First, we excluded periods of eye blinks detected by an on-line parsing system (Eyelink; SR Research Ltd., Ontario). We used a window of 100 ms prior to onsets of eye blinks and 300 ms following their offset in order to minimize after-blink constriction effects. Missing values were linearly interpolated. We adopted pre-processing procedures from Hupé et al. (2009). Specifically, a baseline for each trial was calculated by averaging data points from 500 ms immediately preceding the onset of the stimulus and then subtracting this mean from each trial. The baseline corrected values were z-scored to allow comparison across participants and filtered using a low-pass filter (4 Hz cutoff frequency) to reduce measurement noise. After preprocessing, trials were examined and excluded if they had outliers, exceeding two standard deviations, and no pupillary

light reflex in response to luminosity changes of presented stimuli. The average number of trials excluded for each participant was 19.1% (SD = 9.1). For statistical analysis, the pupillary response was defined as the overall pupil diameter change (i.e., area under curve) within a 1000 ms window, beginning 1 second after stimulus onset. Data for one participant was excluded due to technical problems.

4.5 Results

Self-report and neural measures were evaluated with repeated measures analysis of variance. Pupillary response was evaluated with a mixed linear model in order to account for missing trials across conditions and to increase sensitivity of the analysis. Pairwise comparisons for all measures were made using the Bonferroni adjustment for multiple comparisons.

4.5.1 *Self-reported shock likelihood*

Shock likelihood ratings varied as a function of stimulus type ($F_{(3,72)} = 77.9$, $p < .001$; Figure 3a). Pairwise comparisons showed that shocks were rated as more likely following the CS ($M = 3.9$, $SD = 1$) compared to GS ±20% ($M = 3.02$, $SD = 0.88$; $p = .007$), GS ±40% ($M = 1.66$, $SD = 0.67$; $p < .001$) or GS ±60% ($M = 1$, $SD = 0.14$; $p < .001$). In addition, shocks were rated as more likely following the GS ±20% compared to both GS ±40% ($p < .001$) and GS ±60% ($p < .001$), and the GS ±40% compared to GS ±60% ($p < .001$).

4.5.2 *Pupillary response*

A generalization gradient of pupillary response is presented in Figure 3(b). Pupillary response varied as a function of stimulus type ($F_{(4,44)} = 30.4$, $p < .001$). Pairwise comparisons showed that pupillary response was larger for the $CS_{unpaired}$ versus GS ±40% ($p < .001$) and GS ±60% ($p < .001$). The difference between the $CS_{unpaired}$ and the GS ±20% was marginally

significant (p = .078). Comparisons for GS ±20% versus GS ±40% and GS ±60% and for GS ±40% versus GS ±60% were not significant (all *ps* > .10).

4.5.3 *Gradient of neural reactivity*

Generalization gradients for the right and left insula are presented in Figure 4(c) and Figure 4(b), respectively. Reactivity in both regions varied as a function of stimulus type (right insula: $F_{(3,72)} = 21.8$, $p < .001$; left insula: $F_{(3,72)} = 9.2$, $p < .001$). For the right insula, there was higher reactivity for the $CS_{unpaired}$ versus GS ±40% ($p < .001$) and GS ±60% ($p < .001$), and for the GS ±20% versus GS ±40% ($p < .001$). A comparison of the GS ±20% to GS ±60% was marginally significant ($p = .055$). For the left insula, reactivity was higher for the $CS_{unpaired}$ versus GS ±40% ($p = .002$) and GS ±60% ($p = .04$), and for the GS ±20% versus GS ±40% ($p < .001$) and GS ±60% ($p < .03$). In addition to the insula, the F-contrast revealed significant clusters for the *right supplementary motor area* (SMA), *anterior cingulate cortex* (ACC), *somatosensory cortex, caudate, ventromedial (vmPFC)* and *primary visual cortex*. We therefore, generated gradients for these brain regions in order to compare their response patterns to that of the insula. Reactivity in the ACC (Figure 5a; $F_{(3,72)} = 9.7$, $p < .003$), right SMA (Figure 5b; $F_{(3,72)} = 15.7$, $p < .001$), and the right and left caudate (Figure 5c; right caudate: $F_{(3,72)} = 11.52$, $p < .001$; left caudate: ($F_{(3,72)} = 9.39$, $p < .001$) showed a similar response pattern with higher reactivity associated with increased similarity of GS to CS. For the ACC, reactivity was higher for the $CS_{unpaired}$ and for the GS ±20 relative to GS ±40% ($p < .001$and $p = .001$, respectively). For the right SMA, reactivity was higher for the $CS_{unpaired}$ versus GS ±40 ($p < .001$) and GS ±60% ($p = .001$), and for the GS ±20% versus GS ±40 ($p = .003$) and GS ±60% ($p = .03$). For the right caudate, reactivity was higher for the $CS_{unpaired}$ versus GS ±40 ($p < .001$) and GS ±60% ($p = .04$), and for the GS ±20% versus GS ±40 ($p = .001$). Finally, for the left caudate, reactivity was

higher for the $CS_{unpaired}$ versus GS ±40 ($p < .001$) and GS ±60% ($p = .03$), and for the GS ±20% versus GS ±40 ($p = .01$). In contrast, reactivity in the vmPFC (Figure 6a; $F_{(3,72)} = 13$, $p < .001$) and somatosensory cortex (Figure 6b; $F_{(3,72)} = 13$, $p < .001$) showed a reverse response pattern. For the vmPFC, reactivity was higher for the GS ±60% versus GS ±40 ($p = .01$), GS ±20% ($p < .001$) and $CS_{unpaired}$ ($p < .001$), and for the GS ±40 versus $CS_{unpaired}$ ($p = .02$). Similarly, for the somatosensory cortex, reactivity was higher for the GS ±60% versus GS ±40 ($p = .03$), GS ±20% ($p = .007$) and $CS_{unpaired}$ ($p < .001$), and for the GS ±40 versus $CS_{unpaired}$ ($p = .02$). Finally, there was no significant effect of stimulus type for the right ($F_{(3,72)} = 2.5$, $p = .08$) and left ($F_{(3,72)} = .5$, $p = .66$) visual cortex.

4.5.4 *Direct comparisons of $CS_{unpaired}$ versus GS*

Areas of activation for the $CS_{unpaired}$ versus GS ±40% and GS ±60% are presented in Table 1. This direct comparison showed enhanced activation in the anterior insula, SMA, cingulate gyrus, caudate, thalamus, and frontal areas. Reactivity in these regions is commonly reported in neuroimaging studies of fear learning (Sehlmeyer et al., 2009) and suggests that generalization engages the same circuitry. There was no significant activation for the $CS_{unpaired}$ versus GS ±20% contrast.

Examination of the reverse contrast (i.e., GS ±40% and GS ±60% versus $CS_{unpaired}$; Table 2) showed increased activation in the vmPFC, somatosensory and motor areas, and subcallosal and posterior cingulate. Both the vmPFC and cingulate are associated with modulation of the fear response. There was no significant activation for the GS ±20% versus $CS_{unpaired}$ contrast.

4.5.5 *Amygdala reactivity*

The $CS_{unpaired}$ versus GS comparisons did not reveal significant activation in the amygdala. However, since several studies of fear conditioning have demonstrated habituation in

the amygdala (Büchel et al., 1998; LaBar et al., 1998), we conducted a linear time modulation analysis to test whether amygdala reactivity showed a decline in reactivity over time. For this analysis, we included linear regressors in a new statistical model to account for time effects in reactivity to the $CS_{unpaired}$, GS ±20%, GS ±40% and GS ±60%. There was a significant decrease in amygdala reactivity over time for all four trial groups ($CS_{unpaired}$: Right: x = 32, y = -2, z = -12, $t_{(96)}$ = 4.9, $p <$ .001, Left: x = -26, y = -2, z = -12, $t_{(96)}$ = 3.82, p = .01; GS ±20%: Right: x = 32, y = -2, z = -18, $t_{(96)}$ = 4.12, p = .005; GS ±40%: Right: x = 18, y = 4, z = -18, $t_{(96)}$ = 4.07, p = .03, Left: x = -26, y = 0, z = -16, $t_{(96)}$ = 4.07, p = .006; GS ±60%: Right: x = 26, y = -2, z = -22, $t_{(96)}$ = 3.56, p = .03; small volume corrected with a bilateral amygdala mask and α = .05). There was no interaction of time and stimulus type.

4.6 Discussion

Across two experiments, the right and left insula showed increased activation to the CS and decreases in response amplitude as a function of increasing dissimilarity between the GS and CS. In addition to the insula, the anterior cingulate cortex (ACC) the right supplementary motor area (SMA), and caudate showed a similar reactivity pattern in the second experiment[2]. Reactivity in the ventromedial prefrontal cortex (vmPFC) and primary somatosensory cortex showed the opposite pattern (i.e., largest response to the GS most dissimilar to the CS), and reactivity in the visual cortex was sensitive to increases in stimuli size. Thus, a pattern consistent with autonomic quantifications of generalization was restricted to the insula, ACC, right SMA, and caudate, and was not universally present across the brain. The inverse reactivity pattern in the vmPFC, an area linked to extinction recall and safety learning (Corcoran & Quirk, 2007;

[2] Reactivity patterns for the ACC and SMA in experiment 1 also showed a linear trend but only with a less stringent whole-brain threshold of α = .01.

Milad et al., 2007) may be associated with inhibition of the fear response as GS decrease in similarity to the CS. With respect to the somatosensory cortex, the reduced response in this region during presentation of the $CS_{unpaired}$ may be indicative of participants' preparatory response to expected shocks that are not presented. Similarly, a recent study found deactivation in somatosensory areas for a condition in which shocks were expected but not delivered relative to CS_{minus} (Linnman, Rougemont-Bucking, Beucke, Zeffiro, & Milad, 2011).

The insula is a key structure implicated in anticipatory processing and receives information about the salience, relative value and interoception associated with stimuli (Craig, 2002; Lovero, Simmons, Aron, & Paulus, 2009; Menon & Uddin, 2010). The anterior portion, in particular, is commonly activated in paradigms that elicit autonomic arousal, and is thought to be involved in prediction of future aversive body states linked to conditioned stimuli (Paulus & Stein, 2006), and interoception (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004). Information received by the insula is integrated and relayed to other regions that execute attentional, physiological and motor responses. The anterior insula and ACC are reciprocally connected and co-activate in a range of behaviors, including in preparatory responses to painful stimuli (Craig, 2009; Medford & Critchley, 2010). In the context of the current paradigm, we speculate that the pattern of ACC activation observed may be associated with increases in attention and defensive physiological changes linked to perceived similarity of the GS to the CS, and that SMA reactivity may be associated with the initiation of a motor defense response to potential threat of the shock. The insula may modulate these responses, which is consistent with its proposed role in linking anticipatory processing and autonomic arousal with action-planning aimed at avoidance behavior (Simmons et al., 2006).

The striatum (i.e., caudate and putamen) has been implicated in prediction of both appetitive (O'Doherty, 2004) and aversive (Delgado, Li, Schiller, & Phelps, 2008; Schiller, Levy, Niv, LeDoux, & Phelps, 2008) stimuli. Specifically, studies using shocks (Schiller et al., 2008) and monetary loss (Delgado, Li, et al., 2008) as reinforcers report that activity in this region correlate with prediction error (PE) signals that represent the difference between an actual and an expected outcome. For the generalization task, these PE signals would be hypothesized to gradually decrease as anticipation of a shock diminishes. In addition, the striatum is involved in inhibitory motor control (Vink et al., 2005) and may be associated with inhibition of motor responses to anticipated shocks.

The amygdala did not show activation for the $CS_{unpaired}$ versus GS comparisons. However, we found a decline in amygdala reactivity to the $CS_{unpaired}$, GS ±20%, GS ±40% and GS ±60% overtime. Previous studies of fear conditioning reported habituation in amygdala response with increasing number of trials (Büchel et al., 1998), which supports an amygdala role in learning CS-UCS (unconditioned stimulus) associations in the early stages of conditioning (Bach, Weiskopf, & Dolan, 2011; Büchel et al., 1998; LaBar et al., 1998). Attenuation in amygdala response was also observed when participants were informed about the CS-UCS contingency (i.e., instructed fear paradigm) and was correlated with a physiological expression of fear (Phelps et al., 2001). Animal studies have long demonstrated that the amygdala is involved in both acquisition and expression of fear responses (Davis, 1992; LeDoux, 2000). More recently, plasticity in GABAergic synapses in the lateral (Shaban et al., 2006) and central nuclei (Ciocchi et al., 2010) of the amygdala was linked to generalization of conditioned fear responses. The amygdala response pattern in this task was the same for the $CS_{unpaired}$ and GS and may reflect greater engagement of the amygdala by all stimuli, early on, when the relationship

between stimuli and shock is established. The absence of a differential response for the $CS_{unpaired}$ and GS may be due to their similarity. The process of learning the stimulus-shock associations likely depends on inputs from mPFC- a region involved in conscious threat appraisal (Mechias, Etkin, & Kalisch, 2010). Additionally, the amygdala may be involved in gating the expression of fear responses, as proposed by Ciocchi et al.(2010), possibly via inhibitory inputs from vmPFC. The amygdala and other regions engaged by the generalization task are part of a core neurocircuitry underlying fear conditioning (LeDoux, 2000). Similar regions were implicated in a recent study that examined generalization in response to graded facial expressions (Dunsmoor, Prince, et al., 2011). Together, these findings provide strong evidence that a shared neurocircuitry supports both forms of fear learning.

Neural reactivity in the insula, ACC, right SMA, and caudate paralleled participants' mean post-task ratings of perceived shock likelihood and pupillary response for each stimulus. This reactivity pattern across measures, demonstrating a peak response to the CS and decline in responding associated with greater perceptual dissimilarity of the GS to the CS, was detected despite explicit pre-task instructions regarding the identity of the CS and the reinforcement contingencies to the CS and GS. These findings are consistent with generalization paradigms using fear-potentiated startle, which also showed that healthy individuals primarily generalize defensive physiological responses to GS that are closest in similarity to the CS (Hajcak et al., 2009; Lissek et al., 2008). The convergent validity of these various measures suggests that, used together, they may facilitate assessment of individual variability in the generalization of fear responses, which may help distinguish healthy and clinical populations. In line with preliminary findings in panic disorder, which show a physiological generalization pattern that extends to GS with greater perceptual difference from the CS (Lissek et al., 2010), we expect that anxious

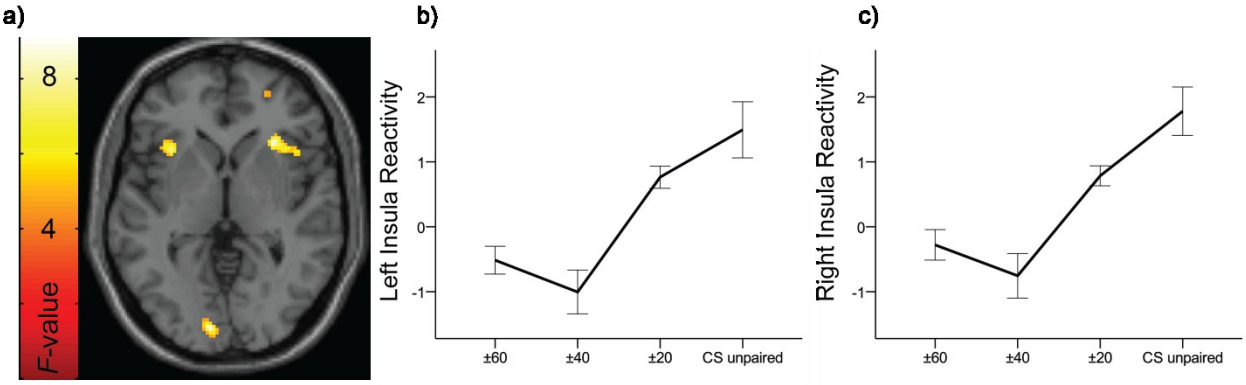
patients, relative to healthy individuals, will demonstrate flatter generalization gradients to more perceptually dissimilar stimuli. Furthermore, based on evidence for vmPFC hypoactivation in PTSD (Etkin & Wager, 2007) it is plausible that the neural gradient in this region may track patients' deficiencies in top-down modulation of the fear response. Together, these gradients may serve as potential markers for diagnosis and/or for prediction of treatment response and therapeutic efficacy. Finally, the inclusion of disorder-specific GS in future studies may help to further discriminate fear responses in different anxiety disorders.

Table 3. Brain activation associated with response to the $CS_{unpaired}$ and generalization stimuli (Healthy group).

Analysis & Region	Hemisphere	MNI Coordinates				Maximum Voxel
		x	*y*	*z*	voxels	*t* value
$CS_{unpaired}$ > GS ±40 & ±60						
Insula	R	32	22	-4	1087	6.55
	L	-32	20	-12	267	4.64
Anterior cingulate	R	10	20	34	64	4.40
Cingulate gyrus (BA 23)	R	4	-22	32	276	4.77
Caudate	R	10	12	8	190	5.14
	L	-12	0	10	199	4.24
Thalamus	L	-8	-14	14	46	3.96
Superior frontal gyrus (including SMA, BA 8)	R	4	36	46	505	5.61
Middle frontal gyrus	R	32	54	-6	215	4.85
BA 40 (extending to BA 7)	R	44	-60	56	608	4.63
	L	-52	-44	56	79	4.44
Middle temporal gyrus	R	56	-28	-6	201	4.69
GS ±40 & ±60 > $CS_{unpaired}$						
Ventromedial prefrontal cortex	R-L	6	34	-18	591	5.37
Subcallosal cingulate	R-L	-4	12	-10	133	5.15
Medial frontal gyrus	L	-4	60	-4	174	4.91
Precentral gyrus (extends to postcentral gyrus)	R-L	8	-30	70	1964	5.58
Posterior cingulate	R	32	-52	22	313	4.65
	L	-6	-58	20	63	4.07
Angular gyrus	R	46	-76	32	474	4.86
	L	-46	-74	8	184	4.03

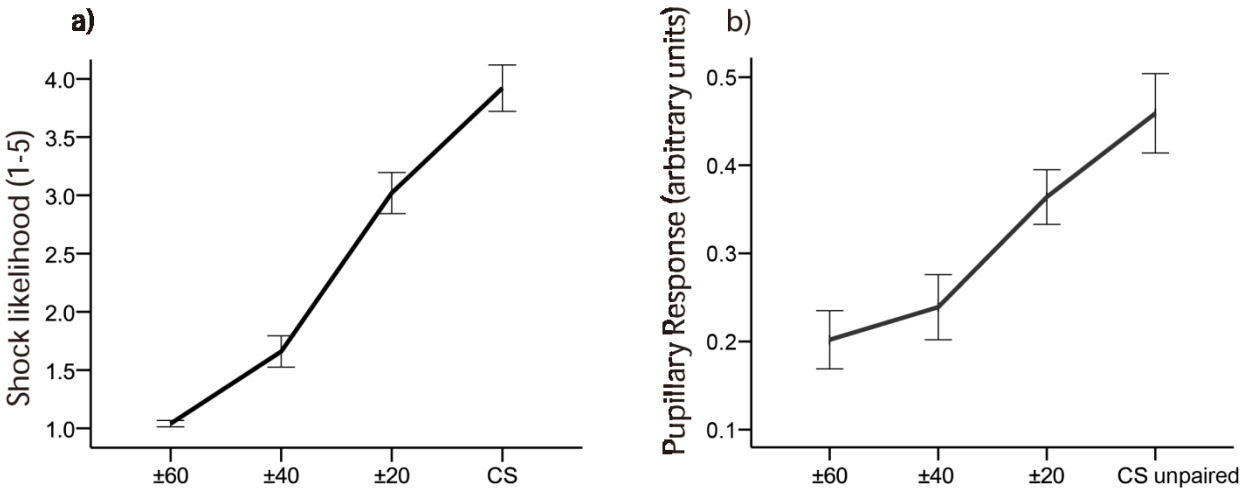
Whole brain threshold $\alpha = .001_{uncorrected}$, extent threshold = 20 voxels; BA = Brodmann area

Figure 2. Activation map and neural gradients for the right and left insula (Pilot study).



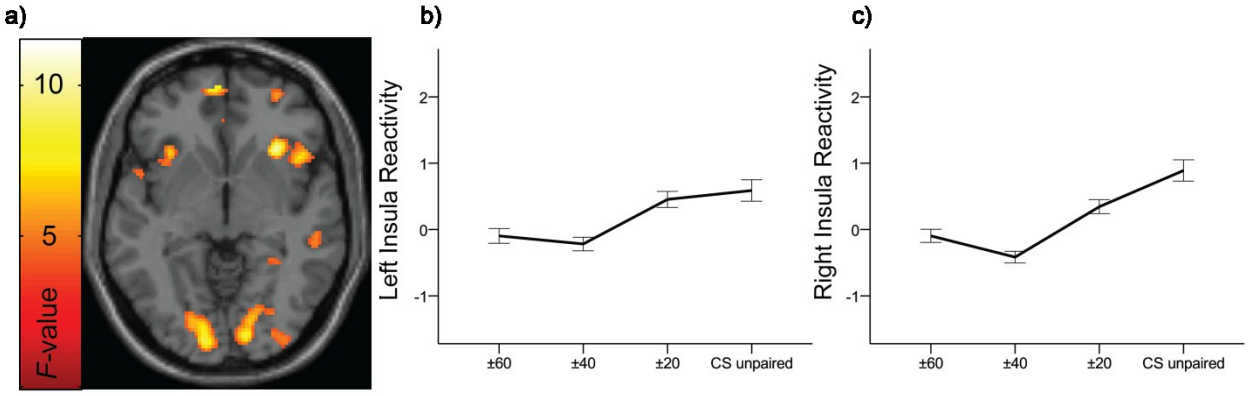
(a) An axial slice shows activation in the right and left insula for the F- contrast main group effect ($P < .001_{uncorrected}$). Left (b) and right (c) insula reactivity as a function of stimulus type. Error bars indicate standard errors of means.

Figure 3. Post-task ratings of shock likelihood and pupillary response as a function of stimulus type (Healthy group).



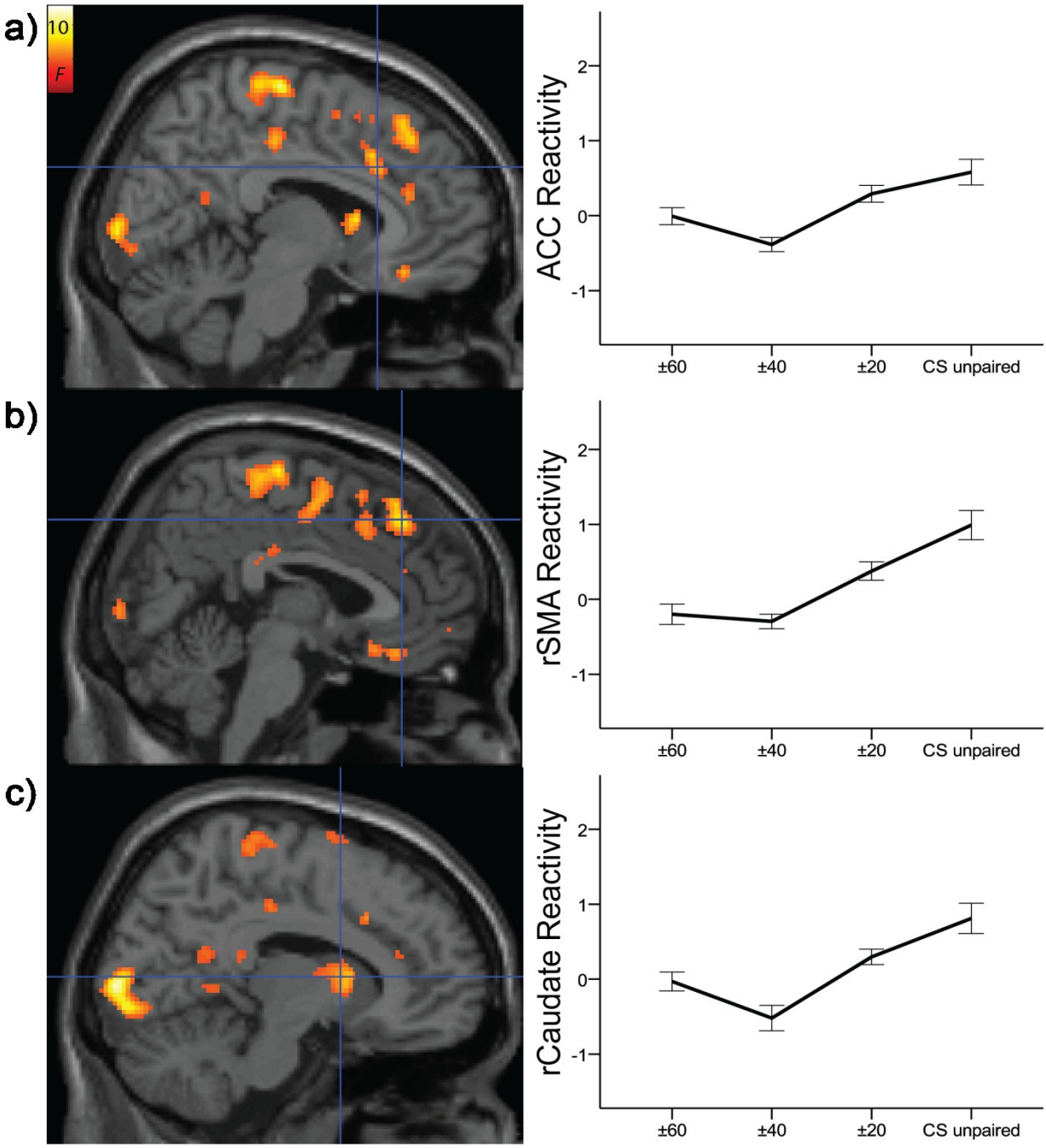
(a) Likelihood of shock was rated on a 5-point Likert scale with 1 = "certainly not shocked" and 5 = "certainly shocked". (b) Pupillary response measures (arbitrary units). Error bars indicate standard errors of means.

Figure 4. Activation maps and neural gradients for the right and left insula (Healthy group).



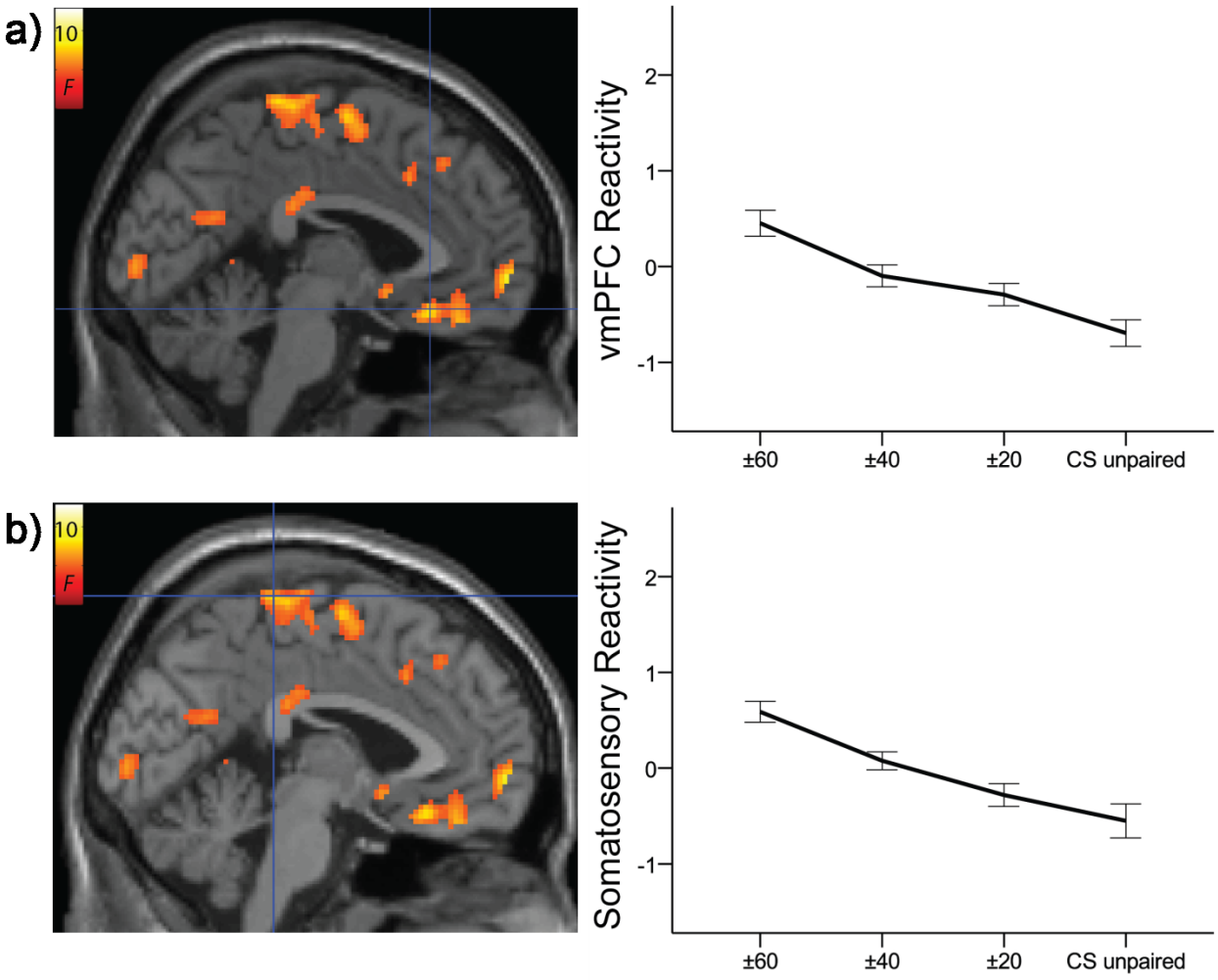
(a) An axial slice shows activation in the right and left insula for the F- contrast main group effect ($P < .001_{\text{uncorrected}}$). Left (b) and right (c) insula reactivity as a function of stimulus type. Error bars indicate standard errors of means.

Figure 5. Activation maps and neural gradients for the anterior cingulate cortex, right supplementary motor area and right caudate (Healthy group).



Sagittal slices showing activation in the anterior cingulate (a), right supplementary motor area (b) and right caudate (c; the gradient for the left caudate was nearly identical and is not presented) for the F- contrast main group effect ($P < .001_{\text{uncorrected}}$) are presented on the left. Cross-hairs indicate the maximum activated voxel within each region used for extraction of the first eigenvariates. Neural gradients for each region are presented on the right. Error bars indicate standard errors of means.

Figure 6. Activation maps and neural gradients for the ventromedial prefrontal cortex (vmPFC) and somatosensory area as a function of stimulus type (Healthy group).



Sagittal slices showing activation in vmPFC (a), and somatosensory area (b) for the F- contrast main group effect ($P < .001$ $_{uncorrected}$) are presented on the left. Cross-hairs indicate the maximum activated voxel within each region used for extraction of the first eigenvariates. Neural gradients for each region are presented on the right. Error bars indicate standard errors of means.

Chapter 5: Ventromedial Prefrontal Cortex Reactivity is Altered in Generalized Anxiety Disorder during Fear Generalization

5.1 Introduction

Fear generalization is the transfer of conditioned fear to perceptually similar stimuli. This process has gained interest in recent years due to its proposed role in the development and maintenance of anxiety symptoms by extending learned fear responses from threat-related stimuli to nonthreatening cues (Lissek et al., 2008). The potential effect of generalization is exemplified in post-traumatic stress disorder (PTSD), in which symptoms of the disorder can be elicited by cues/situations far removed from the initial trauma. Similarly, in generalized anxiety disorder (GAD), generalization may contribute to an increase in the number of cues/events capable of triggering worry – a cardinal symptom of the disorder, which encompasses a broader range of topics and occurs more frequently in patients with GAD relative to healthy individuals (Borkovec et al., 1991).

Studies have begun to examine fear generalization in healthy individuals using fear-potentiated startle (Hajcak et al., 2009; Lissek et al., 2008) and skin conductance (Dunsmoor et al., 2009; Vervliet et al., 2010) to quantify fear responses to a conditioned stimulus (CS) and to generalization stimuli (GS) that vary in perceptual similarity to the CS. The typical response pattern observed in these studies is characterized by a steep linear slope, with a peak fear and physiological response to the CS that declines with increasing dissimilarity of the GS to the CS.

Individuals with panic disorder exhibit flatter fear gradients with more gradual decreases in fear response to the GS (Lissek et al., 2010). In addition, enhanced generalization to conceptually similar stimuli (e.g., spider and spider web) has been shown to correlate with trait anxiety (Dunsmoor, White, et al., 2011). Further evidence for a possible link between deficits in

generalization and anxiety was provided by Hajcak et al. (2009), who reported deficits in a generalization paradigm as a function of variation in the brain-derived neurotrophic factor (BDNF) genotype, which has been related to both learning and anxiety-related behaviors.

In a recent study, we extended this research by examining the neural correlates of generalization in a sample of 25 healthy women (Greenberg et al., 2011). Brain regions engaged by the generalization task included the insula, anterior cingulate cortex (ACC), supplementary motor area (SMA), caudate, amygdala, ventromedial prefrontal cortex (vmPFC) and the somatosensory cortex. The same regions have also been implicated in fear conditioning, which supports a common circuitry for these two processes. Furthermore, neural reactivity in the insula, ACC, SMA and caudate showed a similar response gradient to what has been observed in healthy individuals using peripheral measures of fear. Reactivity in vmPFC and somatosensory cortex, on the other hand, showed an opposite pattern with peak response to stimuli most dissimilar to the CS. These neural gradients suggest that while some brain regions track the fear response to CS and GS, the vmPFC may reflect fear inhibition.

The aim of the current study was to test whether neural gradients observed in our Healthy group differ in GAD. Based on evidence for enhanced fear generalization in anxious individuals (Dunsmoor, White, et al., 2011; Lissek et al., 2010), we hypothesized that patients with GAD would demonstrate flatter slopes for neural gradients relative to healthy individuals. A second aim was to compare connectivity for the GAD and Healthy groups in primary regions engaged by the generalization task. To address our second aim, we conducted psychophysiological interaction (PPI) analysis to examine coupling between the anterior insula (aINS) and: the ACC, SMA, caudate, amygdala, vmPFC and somatosensory cortex as a function of the CS and GS. We selected the aINS as the 'seed' region because of its proposed role in modulating attention,

autonomic reactivity and motor responses, and its relation to anxiety (Paulus & Stein, 2006); this region also showed the most robust activation across participants. We hypothesized that the aINS would show greater coupling with regions implicated in attention and physiological expression of fear during the presentation of the CS. Based on findings of increased insular activation in anxious populations (Paulus & Stein, 2006) we predicted that these PPI effects would be stronger in GAD.

5.2 Method

5.2.1 *Participants*

Thirty-two women with a diagnosis of GAD (50% with and without comorbid major depression; *Mean age overall* = 22.3, *SD* = 4.5) and 25 healthy women (*Mean age* = 21.6, *SD* = 5.1) were included in the analysis. We recruited only women because anxiety is more commonly diagnosed in females, as well as to reduce gender-related heterogeneity in the sample. All participants completed the Structured Clinical Interview for DSM-IV Axis I Disorders - Patient Edition, Version 2 (SCID-I/P; First et al., 2002) to confirm diagnoses of GAD in the patient group and absence of Axis I diagnoses in the Healthy group. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

5.2.2 *Experimental paradigm*

The experimental paradigm is described in chapter 4.

5.2.3 *Self-report questionnaires*

After the fMRI session, participants completed the Trait scale of the State-Trait Anxiety Inventory (STAI-T; Spielberger et al., 1983) and the Beck Depression Inventory (BDI-II; Beck

et al., 1996) to assess continuous measures of self-reported anxiety and depressive symptoms, respectively.

5.2.4 *Gradients of neural reactivity*

We generated neural gradients for all regions previously examined in the Healthy group, including the right and left insula, anterior cingulate cortex (ACC), supplementary motor area (SMA), right and left caudate, ventromedial prefrontal cortex (vmPFC) and somatosensory area by extracting the first eigenvariate (i.e., the principal component) from a 6 mm sphere centered on the local maxima within each region, for each of the '$CS_{unpaired}$ – Baseline' and 'GS – Baseline' contrasts, across all participants. Mean values for $CS_{unpaired}$, as well as GS ±20%, GS ±40% and GS ±60%), were plotted as a four-point gradient.

5.2.5 *Psychophysiological interaction (PPI)*

PPI is a connectivity technique based on regression models; it identifies which voxels within the entire brain (or within regions of interest) show increased coupling with a seed region, in response to specific conditions of a task (see Friston et al., 1997 for details). Here, we conducted two PPI analysis in order to examine connectivity of the right aINS (seed region) with the ACC, SMA, caudate, amygdala, vmPFC and somatosensory cortex as a function of exposure to the CS relative to GS ±60% (first analysis) and the CS relative to all GS combined (second analysis). The psychological (or treatment) vector was 'CS versus GS ±60%' ('CS versus GS ±20% + GS ±40% + GS ±60%' for the second analysis) and the physiological vector, for both analyses, was the first eigenvariate time series extracted from a 6 mm radius sphere centered on the maximally activated voxel in the right aINS for each participant using a height threshold of $p < 0.05$ and extent threshold = 5 contiguous voxels (the search area for the maximally activated voxel for each participant was restricted to a sphere mask [R=6 mm] centered on the peak voxel

in the aINS from the main effect F-contrast of the entire sample). For seven participants (3 GAD and 4 healthy) there were no significant voxels within the seed region at this threshold and these participants were excluded from further analysis. Contrast images from all remaining participants (N = 50) representing the interaction term between the source time-series and treatment vector were used in second-level random effects analyses with a whole-brain threshold of $\alpha = .001_{uncorrected}$ and a small volume familywise error rate corrected $\alpha = .05$, for the ACC, SMA, caudate, amygdala, vmPFC and somatosensory cortex using individual bilateral masks generated with the Masks for Regions of Interest Analysis software (Walter et al., 2003).

5.2.6 *Statistical analyses for behavioral measures and neural gradients*

We assessed self-report ratings of shock likelihood and pupillary response with a 2 (*group: GAD vs. Healthy*) × 4 (*stimulus type: $CS_{unpaired}$, GS ±20%, GS ±40%, GS ±60%*) mixed-model repeated measures analysis of variance and a mixed linear model, respectively. Pairwise comparisons were made with the Bonferroni adjustment for multiple comparisons. We examined group differences in neural gradients slopes with a 2 (*group: GAD vs. Healthy*) × 4 (*stimulus type*) trend analysis for repeated measures; *GAD* (N =32), *Healthy* (N =25). Comparisons between the GAD-only (N = 15) and GAD with comorbid depression (N =17) subgroups did not reveal significant results for either measure (all *ps* > .6).

5.3 Results

5.3.1 *Self-reported shock likelihood and pupillary response*

Shock likelihood ratings varied as a function of stimulus type ($F_{(3,165)} = 108.3$, $p < .001$; see Figure 7). Pairwise comparisons confirmed the impression from Figure 7 that shocks were rated as more likely following the CS ($M = 4.05$, $SD = 1.03$) compared to GS ±20% ($M = 2.87$, $SD = 1.03$; $p < .001$), GS ±40% ($M = 1.77$, $SD = .88$; $p < .001$) and GS ±60% ($M = 1.3$, $SD = .77$;

p < .001). In addition, shocks were rated as more likely following the GS ±20% compared to both GS ±40% (p < .001) and GS ±60% (p < .001), and the GS ±40% compared to GS ±60% (p < .001). The main effect of group and the stimulus by group interaction were not significant (p = .26 and p = .19, respectively).

Pupillary response varied as a function of stimulus type ($F_{(3,4121.16)} = 15.59$, $p < .001$). Pairwise comparisons showed that pupillary response was larger for the $CS_{unpaired}$ versus GS ±20% (p < .001), GS ±40% (p < .001) and GS ±60% (p < .001). Comparisons for GS ±20% versus GS ±40% and GS ±60%, and for GS ±40% versus GS ±60% were not significant (all ps ≥ .09). In addition, the main effect of group and the stimulus by group interaction were not significant (both *ps* ≥ .84).

5.3.2 *Gradients of neural activation*

Reactivity in the insula, ACC, SMA and caudate varied as a function of stimulus type with higher reactivity associated with increased similarity of the GS to the CS (*right insula*: $F_{(3,165)} = 19.18$, $p < .001$; *left insula*: $F_{(3,165)} = 17.72$, $p < .001$; *ACC*: $F_{(3,165)} = 8.26$, $p < .001$; *right SMA*: $F_{(3,165)} = 20.02$, $p < .001$; *right caudate*: $F_{(3,165)} = 14.89$, $p < .001$; *left caudate*: $F_{(3,165)} = 10.59$, $p < .001$; neural gradients for these regions are presented in Figure 8; gradients were similar bilaterally therefore only right-sided gradients are shown). A trend analysis for these regions did not show a significant stimulus type by group interaction (all *ps* > .10) and there was no significant main effect of group (all *ps* > .10). In contrast, reactivity in the vmPFC ($F_{(3,165)} = 15.53$, $p < .001$) and somatosensory cortex ($F_{(3,165)} = 11.5$, $p < .001$) showed a reverse response pattern, with highest response to the GS most dissimilar to the CS. A trend analysis in both regions revealed a significant stimulus type by group interaction (*vmPFC*: $F_{linear\ (1,55)} = 4.3$, $p <$

.05; *somatosensory cortex*: $F_{\text{linear }(1,55)} = 30.54$, $p < .001$) with the GAD group showing flatter slopes across stimuli relative to the Healthy group (Figure 9).

5.3.3 *Continuous measures of anxiety and depression*

Mean STAI-T and BDI scores were higher for the GAD-only (STAI-T: $M = 52.67$, $SD = 10.98$; BDI: $M = 18.33$, $SD = 13.07$) and Comorbid groups (STAI-T: $M = 57.24$, $SD = 8.99$; BDI: $M = 25.88$, $SD = 11.53$) than the Healthy group (STAI-T: $M = 37.52$, $SD = 6.52$; BDI: $M = 4.76$, $SD = 3.54$; both $ps < .001$). There were no significant differences in STAI-T scores between the GAD-only and Comorbid groups ($p = .21$). However, there was a trend for higher BDI scores in the Comorbid group ($p = .08$).

We used Pearson correlations to examine whether the slope of vmPFC gradients is associated with levels of trait anxiety and depressive symptoms. Both STAI-T and BDI scores were positively correlated with slope coefficients of individual vmPFC gradients ($r = .33$, $p = .01$ and $r = .39$, $p = .003$, respectively; see Figure 10). Independent correlation analyses in the GAD (N = 32) and Healthy groups showed that these associations were only significant for the GAD group (both $ps < .04$).

5.3.4 *PPI analyses*

Across participants (N = 50), PPI results indicated that, during presentation of the CS relative to the GS ±60%, activity in the right aINS showed greater covariance with activity in the posterior insula (pINS), ACC, SMA and the amygdala (Figure 11). For the second analysis, in which we compared connectivity during the CS relative to all GS, activity in the aINS showed greater coupling with activity in the pINS and the amygdala (Figure 12). There were no significant PPI effects for the opposite contrasts (i.e., during presentation of the GS ±60%,or GS

combined, relative to the CS). We did not find any differences in PPI effects between the GAD group and the Healthy group.

5.4 Discussion

The GAD and Healthy groups exhibited similar neural gradients in the insula, ACC, SMA and caudate – demonstrating an enhanced response to the CS and a decrease in response amplitude as GS were more dissimilar to the CS. In the vmPFC and somatosensory area, reactivity across groups showed a reversed pattern (i.e., largest response to the GS most dissimilar to the CS); within both regions, the GAD group exhibited flatter neural gradients that suggest less differential response across stimuli.

The vmPFC has been implicated in attenuation of fear responses in both animals and humans. Animal studies have shown that lesions in vmPFC regions impair extinction recall (Morgan & LeDoux, 1995; Quirk, Russo, Barron, & Lebron, 2000). In humans, fMRI studies have reported correlations between vmPFC activation and magnitude of extinction memory (Milad et al., 2007; Phelps et al., 2004). Additional evidence for the role of the PFC in controlling fear comes from emotion regulation studies in which participants are instructed to reappraise aversive stimuli in order to reduce their negative impact (Ochsner & Gross, 2005). These studies have reported increased activation in lateral PFC during down-regulation of negative images with corresponding decreases of activation in the amygdala (Ochsner, Bunge, Gross, & Gabrieli, 2002; Urry et al., 2006). A comparison of PFC function during emotion regulation and extinction suggests that during reappraisal the lateral PFC inhibits the amygdala via a shared circuitry with extinction that involves the vmPFC (Delgado, Nearing, Ledoux, & Phelps, 2008).

Neurocircuitry-based models of anxiety disorders have hypothesized hyper-responsivity to threat within the amygdala and deficient vmPFC function mediating inadequate regulation of fear responses (Kent & Rauch, 2003; Rauch, Shin, & Wright, 2003). These response patterns have been demonstrated most consistently in PTSD (Etkin & Wager, 2007; Shin & Liberzon, 2010). For example, patients with PTSD, relative to healthy individuals, exhibit decreased activation in the vmPFC and increased activation in the amygdala in response to symptom provocation paradigms with trauma-related cues (Lanius et al., 2001; Shin et al., 2004). Reduced activation of vmPFC in PTSD has also been found during extinction recall (Bremner et al., 2005; Milad et al., 2009) and was associated with symptom severity (Kim et al., 2008; Shin et al., 2005). In GAD, findings suggest dysfunction in the amygdala-frontal circuitry as well (Blair et al., 2008; Monk et al., 2006; Monk et al., 2008), however, the role of these structures in the pathophysiology of GAD remains poorly understood due to the small number of neuroimaging studies that have focused on this disorder, mixed results from existing studies, and lack of data from paradigms of fear learning (Shin & Liberzon, 2010). Nevertheless, a recent study demonstrated that patients with GAD fail to activate the pregenual ACC (a subsection of the vmPFC) during implicit regulation of emotional conflict (Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010). The vmPFC response pattern observed in the current study suggests that recruitment of vmPFC may also be deficient in GAD when inhibition of fear responses is required (i.e., during presentation of "safe" stimuli). Across GS, the GAD group exhibited less discriminant vmPFC responses as evident by a flatter vmPFC gradient. Furthermore, slope coefficients of patients' individual vmPFC gradients were positively correlated with levels of trait anxiety and depressive symptoms. Healthy individuals, on the other hand, showed greater increases in vmPFC reactivity to consecutive stimuli (reflected by a steeper vmPFC gradient)

and their vmPFC slope coefficients were not associated with measures of anxiety and depression. A failure to properly engage the vmPFC in the presence of stimuli that resemble a CS could facilitate fear generalization and might reflect a broader dysfunction of regulatory skills in GAD, in accordance with its diagnostic criteria pertaining to difficulties in controlling worry and anxiety.

Reactivity in the somatosensory cortex was also less discriminate in the GAD group compared to the Healthy group. Based on findings of deactivation in somatosensory areas when shocks were expected, but not delivered (Linnman et al., 2011), we previously hypothesized that reactivity in this region may be associated with participants' expectation of shocks that are not presented. Accordingly, the response pattern exhibited by the GAD group in this region may be indicative of less certainty regarding stimulus-shock contingencies. Although post-task ratings of shock likelihood were similar for the two groups, this interpretation is supported by greater variability in scores for the GAD group[3].

Across participants, PPI analysis revealed increased right aINS coupling with the right pINS, ACC, amygdala and SMA during presentation of the CS relative to the GS ±60%. The insula is highly interconnected with other brain regions and receives extensive somatosensory and visceral input (Augustine, 1996) as well as information regarding the saliency and value of incoming stimuli (Paulus & Stein, 2006). The aINS, in particular, has been proposed as a hub where this input is integrated and then relayed to other areas in order to guide behavior (Menon & Uddin, 2010). The PPI results are consistent with such a modulatory role for the aINS and suggest that it may facilitate fear response to the CS via the ACC, which is involved in initiation of autonomic and motor responses and has heavy projections to the amygdala and SMA

[3] Levene's test for equality of variances between the two groups was significant for the GS ±60% ($p < .001$) and GS ±40% ($p = .02$).

(Medford & Critchley, 2010). Differences in connectivity during presentation of the CS relative to all the GS combined were less pronounced showing increased right aINS coupling with the right pINS and left amygdala.

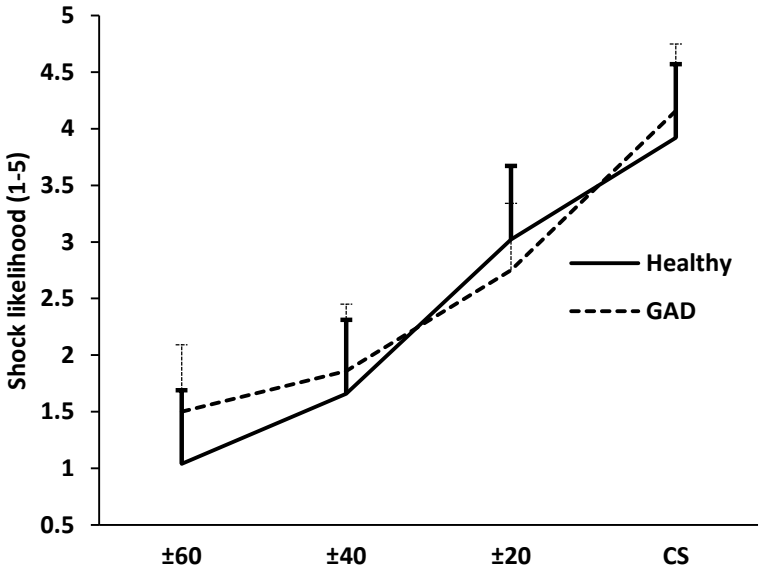
The GAD and Healthy groups did not differ in insular connectivity with the ACC, SMA and amygdala during presentation of the CS. Reactivity in these regions, as well as pupillary responses across stimuli were also the same for the two groups. These results indicate that the strength of the fear response, and its underlying circuitry, were comparable in patients and healthy individuals.

Because participants in this study were young with a relatively short history of symptoms, future studies should examine whether GAD patients with longer illness durations present other alterations in neural reactivity during fear generalization. The current study, like most studies of fear generalization, utilized GS that changed along a physically neutral stimulus dimension (i.e., size). Dunsmoor et al. (2009) proposed that fear-relevant attributes of the GS (e.g., intensity of fear expression) may increase generalization. Neural gradients to such stimuli, including disorder-specific GS, may better distinguish anxious patients and healthy individuals. These types of stimuli will be easier to test in phobia and PTSD where the fear response is triggered by more specific objects/events.

In conclusion, we demonstrate altered vmPFC reactivity in individuals with GAD suggestive of deficient vmPFC recruitment during fear inhibition of GS. Connectivity analyses across participants implicated the aINS in facilitating the fear response to the CS. The GAD and Healthy group did not diverge in insular connectivity or in neural and physiological measures of fear. Thus, these findings suggest that deficits in fear regulation, rather than in the excitatory

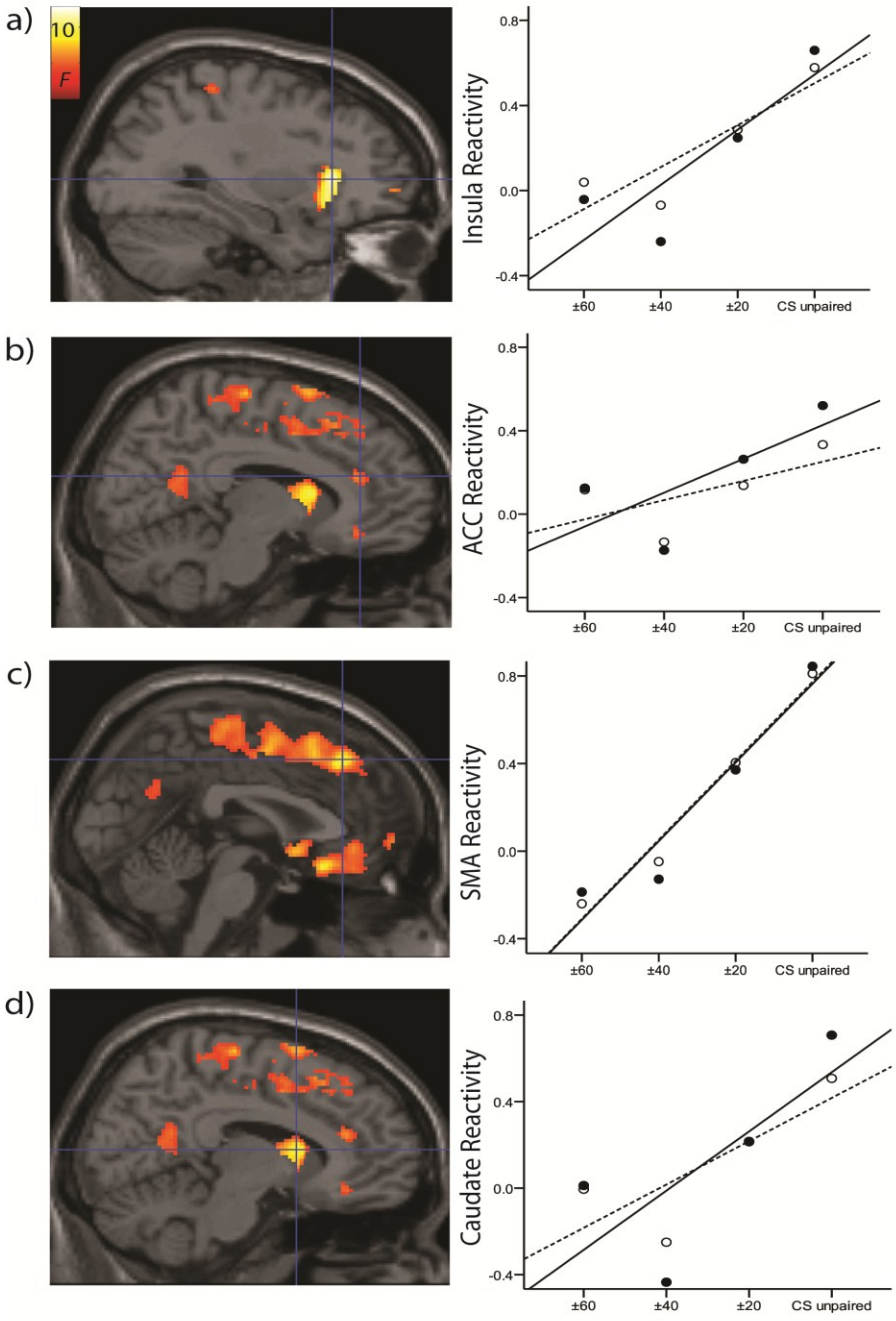
response itself, are more critical to the pathophysiology of GAD in the context of fear generalization.

Figure 7. Post-task ratings of shock likelihood for the GAD (N=32) and Healthy groups as a function of stimulus type.



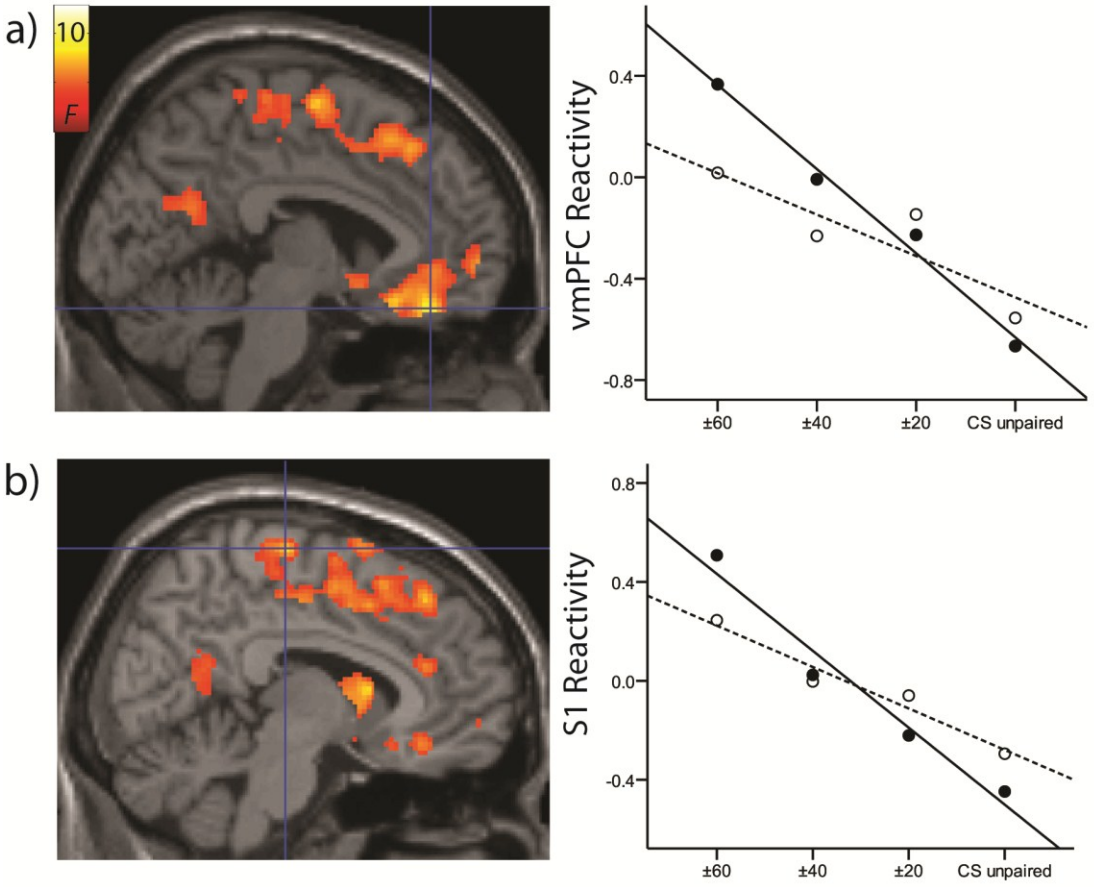
Across groups, delivery of shocks was perceived as being more likely as stimuli became more perceptually similar to the CS. Likelihood of shock was rated on a 5-point Likert-type scale with 1 = "certainly not shocked" and 5 = "certainly shocked".

Figure 8. Activation maps and neural gradients for the right insula, anterior cingulate cortex (ACC), right supplementary motor area (SMA) and right caudate as a function of stimulus type, for the GAD (N= 32) and Healthy groups.



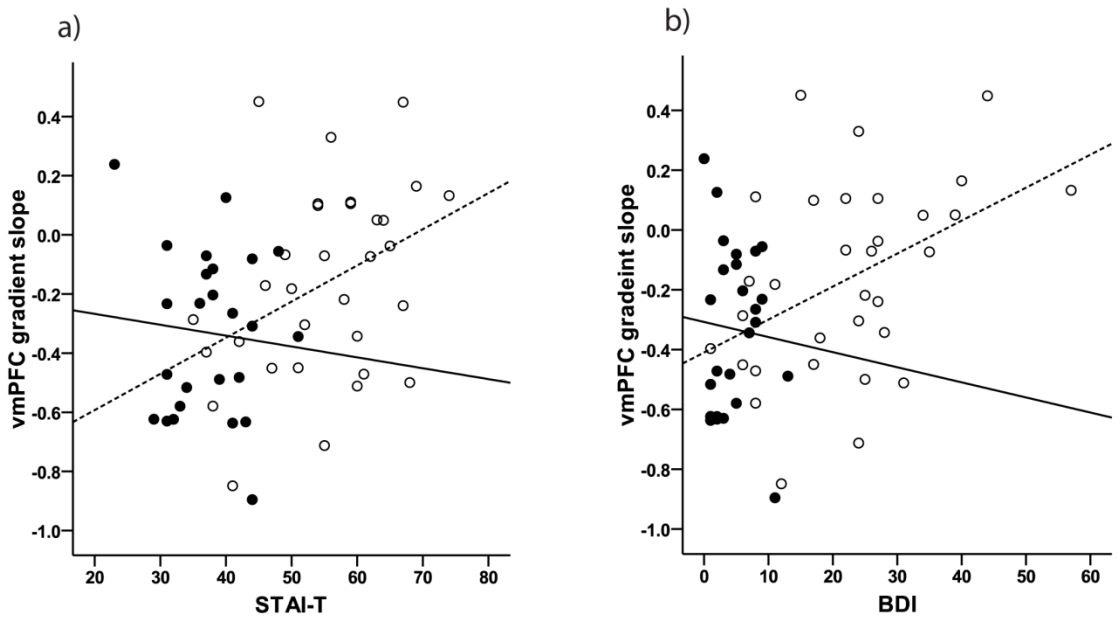
For all four regions, neural gradients for both groups showed similar linear trends. Sagittal slices showing activation in the right insula (a), ACC (b), right SMA (c) and right caudate (d) are presented on the left. Cross-hairs indicate the maximum activated voxel within each region used for extraction of the first eigenvariates (coordinates for right insula = 32, 24, 4; ACC = 10, 36, 20; SMA = 2, 34, 46; and right caudate = 10, 10, 10). Neural gradients and regression slopes in each region are presented on the right for each group; (GAD = unfilled circles/dotted line; Healthy = filled circles/solid line).

Figure 9. Activation maps and neural gradients in the ventromedial prefrontal cortex (vmPFC) and right somatosensory area (S1) as a function of stimulus type for the GAD (N=32) and Healthy groups.



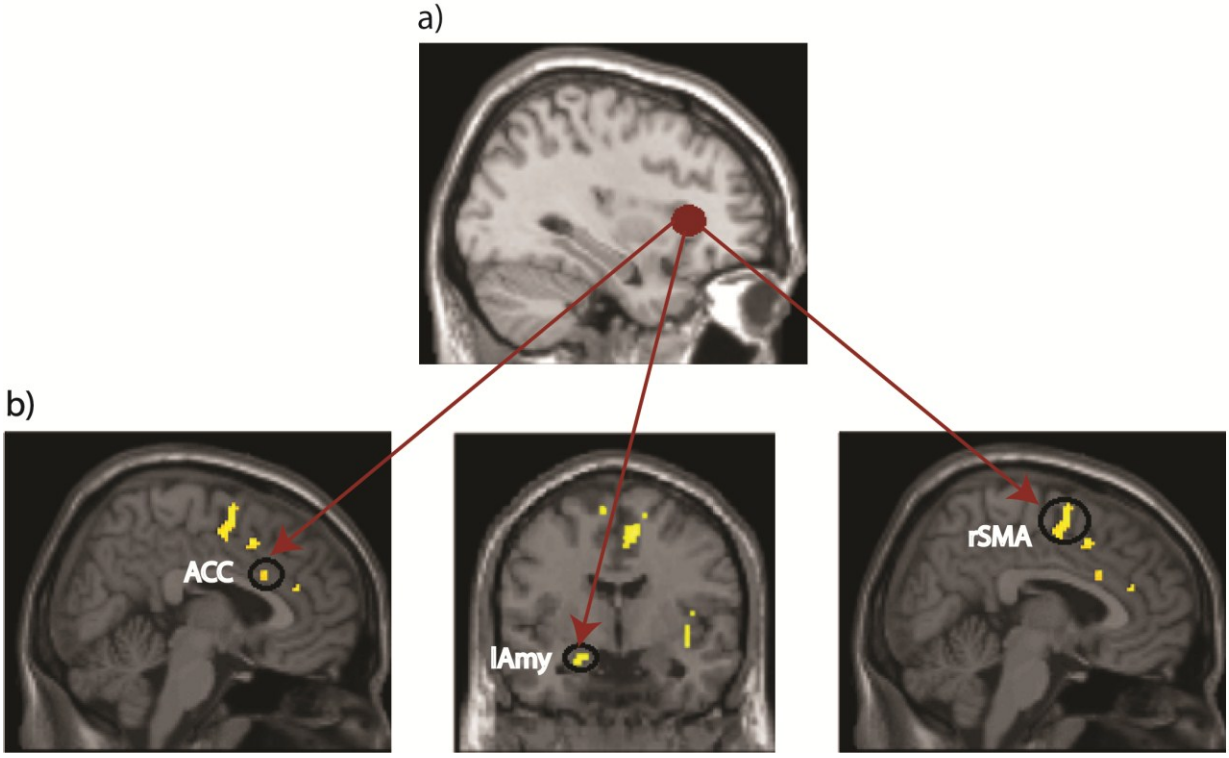
For both regions, the GAD group exhibited flatter slopes for neural gradients compared to the Healthy group. Sagittal slices showing activation in vmPFC (a) and right somatosensory area (S1;b) are presented on the left. Cross-hairs indicate the maximum activated voxel within each region used for extraction of the first eigenvariates (coordinates for vmPFC = -4, 40, -20 and for right somatosensory area = 8, -32, 70). Neural gradients and regression slopes in each region are presented on the right for each group; (GAD = unfilled circles/dotted line; Healthy = filled circles/solid line).

Figure 10. Bivariate correlations between STAI-T (a) and BDI (b) scores and slope coefficients of individual vmPFC gradients for the GAD (N=32) and Healthy groups.



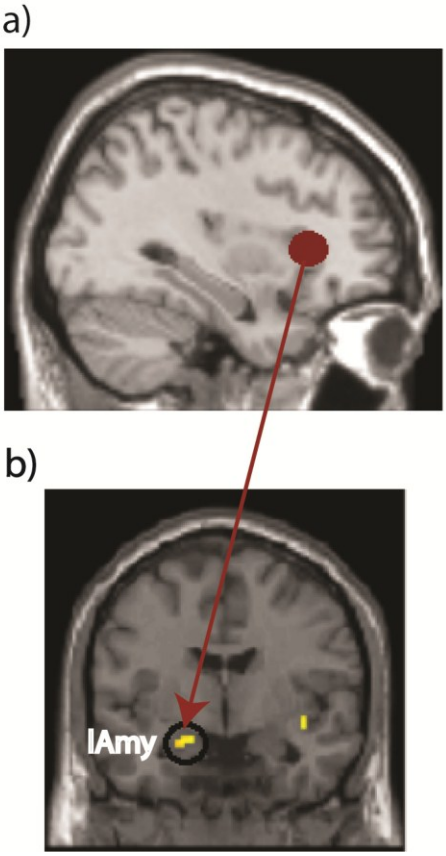
GAD: STAI-T: $r = .37$, $p = .039$; BDI: $r = .42$, $p = .017$; unfilled circles/dotted line
Healthy: STAI-T: $r = -.09$, $p = .68$; BDI: $r = -.06$, $p = .76$; filled circles/solid line

Figure 11. Psychophysiological interactions (N=50) for the right anterior insula seed during presentation of the CS relative to the GS ±60%.



(a) Right anterior insula (aINS) seed. (b) Positive connectivity with the anterior cingulate cortex (ACC), left amygdala (lAmy) and right supplementary motor area (rSMA).

Figure 12. Psychophysiological interactions (N=50) for the right anterior insula seed during presentation of the CS relative to all GS (i.e., GS ±20%, GS ±40% and GS ±60%).



(a) Right anterior insula (aINS) seed. (b) Positive connectivity with the left amygdala (lAmy).

Chapter 6: Anticipation of High Arousal Aversive and Positive Movie Clips Engages Common and Distinct Neural Substrates

6.1 Introduction

Exaggerated anticipation of threatening situations is a central component of anxiety disorders. Patients often experience high levels of anxiety prior to actual exposure to feared stimuli/situations. The neural correlates of anticipation, in relation to anxiety, have been investigated with generally aversive pictures (Nitschke et al., 2006; Nitschke et al., 2009), phobogenic images (Simmons et al., 2004; Simmons et al., 2006; Straube, Mentzel, & Miltner, 2007) and noxious tactile stimuli (Chua, Krams, Toni, Passingham, & Dolan, 1999). The general paradigm used in these studies includes the following components: participants are instructed of cue-stimulus pairings in which one cue is predictive of a negative stimulus and a second cue is predictive of a control or neutral stimulus. The cue is followed by a brief anticipation period (during which participants passively focus on the screen or perform a distractor task). Next, the stimulus associated with the cue is presented. Across studies, insula activation has been most consistently observed during aversive anticipation. Additional regions implicated are the anterior cingulate cortex (ACC), amygdala and prefrontal regions including the lateral prefrontal cortex (lPFC) and medial prefrontal cortex (mPFC).

In contrast to previous studies that assessed short anticipatory periods (Nitschke et al., 2006; Simmons et al., 2004; Simmons et al., 2006), we recently used a modified task with longer anticipation periods (16 seconds) to investigate whether the same regions are engaged when the anticipated event is more distal and to examine the temporal dynamics of the anticipatory response. The task also included a self-report measure at the end of each trial to gauge participants' subjective experience of anticipatory anxiety and its association with neural

reactivity. We ran two versions of the task in healthy individuals, one with loud and soft auditory stimuli (Carlson, Greenberg, Rubin, & Mujica-Parodi, 2011) and a second with negative and neutral images from the international affective picture system (IAPS; Carlson & Mujica-Parodi, 2010). Results for the auditory version largely replicated previous findings, revealing activation in the insula, amygdala, brainstem and superior frontal gyrus during anticipation of loud (100 dB) versus soft (55 dB) white noise. In addition, the right anterior insula (aINS) was associated with participants' self-reported anxiety across trials, and the amygdala was selectively associated with feelings of anxiety during aversive trials. In the pictorial version, anticipation of negative versus neutral images engaged fewer brain regions including the insula, occipitotemporal visual areas and dlPFC and the amplitude and spatial extent of activation was smaller compared to the auditory task (observed only with a liberal threshold of $\alpha = .005_{\text{uncorrected}}$). Participants' self-report ratings of anticipatory anxiety during the visual task were low as well. These findings suggest that affective pictures may not be optimal for eliciting a strong anticipatory response in healthy individuals, particularly for extended durations.

The aim of the present study was to establish a normative sustained anticipatory response for high arousal aversive and positive stimuli and a condition of uncertainty. For this purpose, we made several changes to our previous task. Based on our findings of a restricted anticipatory response to affective pictures, we replaced these stimuli with short movie clips (without sound) known to elicit a strong emotional response (Gross & Levenson, 1995). The movie clips are also more suitable than auditory stimuli for the scanner environment and for manipulating valence and arousal. We also added two conditions, *positive anticipation* in which participants anticipate positive clips and '*uncertain' anticipation* in which they anticipate aversive or positive clips with unknown probability (the positive clips depicted sexual scenes and the aversive clips depicted a

graphic medical procedure); the other conditions were *'aversive anticipation'* and *'neutral anticipation'*. The inclusion of a high arousal positive condition, to contrast the aversive condition, is important for assessing the effect of valence on anticipation while controlling for levels of arousal. Previous studies that compared a high arousal aversive condition to a low arousal neutral condition may have confounded valence with arousal. The 'uncertain' condition was included to examine the effect of uncertainty on anticipation. Lastly, we incorporated pupillary response measures to assess arousal levels during the anticipatory phase.

We hypothesized that anticipation of aversive, positive and 'uncertain' clips would recruit common regions involved in preparatory responses to affective stimuli and that additional brain regions associated with processing appetitive stimuli such as the nucleus accumbens (NAcc) and mPFC would be preferentially engaged during anticipation of positive clips. The insula, which has been previously implicated in processing disgust, was expected to be preferentially engaged during anticipation of aversive clips. Based on evidence for a negativity bias in neural response when anticipating events of unknown valence (i.e., either negative or positive; Herwig, Kaffenberger, Baumgartner, & Jäncke, 2007), we further hypothesized that anticipation of 'uncertain' clips would recruit the same regions activated during aversive anticipation.

6.2 Method

6.2.1 *Participants*

Twenty-five healthy women participated in the study (*Mean age* = 21.6; *SD* = 5.1). Participants were screened for absence of psychiatric illness with the Structured Clinical Interview for DSM-IV Axis I Disorders -Patient Edition, Version 2 (SCID-I/P; First et al., 2002).

The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

6.2.2 *Task Design*

The task consisted of 40 trials presented pseudorandomly: 10 aversive trials (anticipation of an aversive movie clip), 10 positive trials (anticipation of a positive movie clip), 10 'uncertain' trials (anticipation of either a positive or aversive movie clip; 50% each) and 10 neutral trials (anticipation of a neutral movie clip). Each trial began with a white fixation crosshair presented in the center of a black screen (jittered 10 - 14 seconds). The fixation crosshair was followed by an anticipation period during which a countdown from 16 to 1 was numerically presented in the center of the screen with a specific cue associated with each trial type presented above it (16 seconds duration). The cues were: "▼" for a negative clip, "▲" for a positive clip, "**?**" for an 'uncertain' clip and "**—**" for a neutral clip. A movie clip was presented next (6 seconds) and then participants rated the strength of their anticipatory response during the countdown period on a 4-point scale (4 seconds). The duration of the task was 25 minutes and 30 seconds.

6.2.3 *Stimuli*

The aversive clips were taken from a non-commercial surgery film of arm amputation shown to reliably elicit disgust (Gross & Levenson, 1995) and a similar film of a thigh surgery found online. The positive clips were sexually explicit and taken from two scenes featured in Eyes of Desire 2 (Femme Productions) and Island Fever 2 (Digital Playground), which were pilot rated high on arousal and positive valence by women (N=8). The neutral clips were taken from a scene in the movie *Quiet Earth* (1985) showing a man driving.

6.2.4 *Post-task rating of clips*

Outside the scanner immediately following the task, participants rated each clip for valence and arousal using two 9-point scales. The valence scale ranged from 1 = "negative" to 9 = "positive". The arousal scale ranged from 1 = "calm" to 9 = "excited" (participants were instructed that a high score on this scale could reflect either negative or positive arousal).

6.2.5 *Image analysis*

First-level single subject statistical parameter maps were created for the *aversive anticipation, positive anticipation, 'uncertain' anticipation, neutral anticipation*, *aversive clip, positive clip, 'uncertain' clip and neutral clip*. These contrasts were used in a 2 (process: *anticipation, clip presentation*) × 4 (valence: *aversive, positive, 'uncertain', neutral*) second-level random effects repeated measures analysis. In addition, we conducted conjunction analyses, detailed below, using the minimum statistic compared to the conjunction null (Nichols, Brett, Andersson, Wager, & Poline, 2005).

6.3 Results

6.3.1 *Self-report ratings of anticipatory response*

Data for one participant was not available due to technical difficulties. A repeated measures analysis of variance (ANOVA) revealed differences in self-reported anticipatory ratings across conditions ($F_{(3,69)} = 68.75$, $p < .001$). As shown in Figure 13, anticipatory ratings for the aversive ($M = 3.06$; $SD = .71$), positive ($M = 2.54$; $SD = .79$) and 'uncertain' ($M = 2.8$; $SD = .64$) conditions were all higher than the ratings for the neutral condition (all $ps < .001$). These results confirm that, on average, participants experienced a stronger emotional response in anticipation of the affective clips. Anticipatory ratings for the aversive condition were marginally

higher than the ratings for the positive condition (p = .055). There were no significant differences in ratings between the 'uncertain' condition and either the aversive or positive conditions (both $ps \geq .13$).

6.3.2 *Post-task valence and arousal ratings for the movie clips*

Ratings for two participants were not available due to software malfunction. Mean valence and arousal scores for the aversive, positive, 'uncertain'-aversive, 'uncertain'-positive and neutral clips are shown in figures 14 and 15, respectively. Valence scores were highest for the positive (M = 5.83; SD = 1.17) and 'uncertain'-positive clips (M = 5.86; SD = 1.31) and lowest for the aversive (M = 2.93; SD = 1.45) and 'uncertain'-aversive clips (M = 3.11; SD = 1.58). Valence scores for the neural clips were 5.08 (SD = .77). Arousal scores for the aversive (M = 5.77; SD = 2.08), 'uncertain'-aversive (M = 5.40; SD = 2.14), positive (M = 5.36; SD = 1.58) and 'uncertain'-positive clips 5.67 (SD = 1.87) did not differ (all $ps \geq .16$) and were all higher than arousal scores for the neutral clips (M = 1.46; SD = .62; all $ps < .001$).

6.3.3 *Brain activation during anticipation of affective clips*

Comparisons of activation during aversive, positive and 'uncertain' anticipation versus neutral anticipation are presented in table 4. The *aversive anticipation > neutral anticipation* contrast revealed activation in the anterior and mid-insula, thalamus, caudate, ACC, lateral prefrontal cortex (lPFC) and inferior parietal area (BA40). The *positive anticipation > neutral anticipation* contrast revealed activation in the anterior insula (aINS), thalamus, caudate, putamen, nucleus accumbens (NAcc), amygdala, ACC (including supracallosal and perigenual sectors), medial prefrontal cortex (mPFC), lPFC, inferior parietal area (BA40), superior temporal gyrus and midbrain. The '*uncertain' anticipation > neutral anticipation* contrast revealed

activity in the aINS, thalamus, caudate, NAcc, amygdala, ACC, mPFC, lPFC, middle temporal gyrus, inferior parietal area (BA 40) and midbrain.

6.3.4 *Conjunction analyses for the anticipation contrasts*

In order to identify regions commonly activated for the aversive anticipation, positive anticipation and 'uncertain' anticipation (versus neutral anticipation) we conducted a conjunction analysis for these three contrasts. This analysis revealed activation in the aINS, thalamus, caudate, dorsal anterior cingulate cortex (dACC), lPFC, and inferior parietal area (BA 40; see Figure 16).

To identify regions commonly activated for 'uncertain' and aversive anticipation we conducted a conjunction analysis using the following four contrasts (adapted from Herwig et al., 2007): 1) '*uncertain' anticipation > neutral anticipation* 2) '*uncertain' anticipation > positive anticipation* 3) *aversive anticipation > neutral anticipation* 4) *aversive anticipation > positive anticipation*. This analysis revealed activation in the right mid-insula. Similarly, to identify regions commonly activated for 'uncertain' and positive anticipation we conducted a conjunction analysis with these four contrasts: 1) '*uncertain' anticipation > neutral anticipation* 2) '*uncertain' anticipation > aversive anticipation* 3) *positive anticipation > neutral anticipation* 4) *positive anticipation > aversive anticipation* (all $ps < 0.001_{\text{uncorrected}}$). This analysis revealed activation in the NAcc and mPFC. There was no significant activation for the *aversive anticipation > 'uncertain' anticipation* and the *positive anticipation > 'uncertain' anticipation* contrasts.

6.3.5 *Differential activation for aversive and positive anticipation*

To identify regions that were selectively activated during aversive anticipation we conducted a conjunction analysis using the *aversive anticipation > neutral anticipation* and

aversive anticipation > *positive anticipation* contrasts. In addition, we applied exclusive masking with the *positive anticipation* > *neutral anticipation* contrast to remove any voxels that were significant for this contrast from the final results. This analysis revealed activity in the right mid-insula (see Figure 17b).

To identify regions that were selectively activated during positive anticipation we conducted a conjunction analysis using the *positive anticipation* > *neutral anticipation* and *positive anticipation* > *aversive anticipation* contrasts. Results were exclusively masked with the *aversive anticipation* > *neutral anticipation* contrast. This analysis revealed activity in the left NAcc and mPFC (see Figure 17a).

6.3.6 *Brain activation in response to the clips*

Regions activated for the *aversive clip* > *neutral clip* contrast included the insula (both anterior and mid-posterior regions), thalamus, dACC, globus pallidus, bilateral precentral gyrus (BA 6), bilateral fusiform gyrus, superior and inferior parietal areas, precuneus (BA 7) and cerebellum. Regions activated for the *positive clip* > *neutral clip* contrast included the aINS, cingulate gyrus (including dorsal and anterior sections), NAcc, caudate, mPFC, thalamus, amygdala, parahippocampal gyrus (BA 34), bilateral fusiform gyrus, superior and inferior parietal areas, precuneus (BA 7), midbrain and cerebellum. Regions activated for the '*uncertain' clip* > *neutral clip* contrast included the aINS, cingulate cortex (including dorsal and anterior sections), NAcc, amygdala, mPFC, bilateral fusiform gyrus, superior and inferior parietal areas, precuneus (BA 7) and cerebellum.

6.3.7 *Differential activation for the aversive and positive clips*

To identify regions that were selectively activated in response to the aversive clips, we conducted a conjunction analysis using the *aversive clip* > *neutral clip* and *aversive clip* >

positive clip contrasts. In addition, we applied exclusive masking with the *positive clip* > *neutral clip* contrast. This analysis revealed activity in the bilateral mid-posterior insula, precentral gyrus (BA 4) and a subsection of the left fusiform area. To identify regions that were selectively activated in response to the positive clips we conducted a conjunction analysis using the *positive clip* > *neutral clip* and *positive clip* > *aversive clip* contrasts. Results were exclusively masked with the *aversive clip* > *neutral clip* contrast. This analysis revealed activity in the NAcc, mPFC, amygdala, medial thalamus, superior temporal gyrus and precuneus (BA7; all *ps* < $0.001_{\text{uncorrected}}$).

6.3.8 *Interaction of period and valence*

In order to compare reactivity during anticipation and presentation of the clips we generated contrast files for the interaction effect of period (anticipation, clip) × valence (affect, neutral) for each affective condition and then conducted conjunction analyses using these files. Regions preferentially engaged during anticipation included the right superior frontal gyrus, lPFC and inferior parietal area (BA 40; observed with inclusive masking using '*anticipation* > *clip presentation'* contrast; see Figure 18a). Regions preferentially engaged during clip presentation were the bilateral fusiform area, superior parietal area and ventral cerebellum (observed with inclusive masking using '*clip presentation* > *anticipation'* contrast; see Figure 18b).

6.4 Discussion

Anticipation of aversive, positive and 'uncertain' clips engaged common regions consisting of the bilateral anterior insula (aINS), thalamus, bilateral caudate, dorsal anterior cingulate cortex (dACC), lateral prefrontal cortex (lPFC; including clusters in superior, middle

and inferior frontal gyri) and bilateral inferior parietal area (BA 40). The nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC), on the other hand, were selectively activated during anticipation of positive clips and the mid-insula was selectively activated during aversive anticipation; all three regions were also activated in the 'uncertain' condition in which either aversive or positive clips were forthcoming. These results suggest that a common circuitry is recruited in anticipation of affective clips regardless of valence, with additional areas preferentially engaged depending on whether expected stimuli are negative or positive.

Recruitment of common regions across affective conditions supports their involvement in a general preparatory response, which likely includes multiple processes such as vigilance, appraisal of upcoming stimuli and one's response to them (i.e., self-referential processing), generation of an affective state and initiation of regulatory mechanisms. The aINS has been frequently implicated in aversive anticipation; reactivity in this region across affective conditions is consistent with its broader role in processing and awareness of interoceptive signals associated with salient stimuli (Craig, 2002; Menon & Uddin, 2010). This information is relayed to other regions such as the dACC and lPFC where it can modulate attentional and regulatory processes and is also thought to contribute to the rise of conscious feeling states (Craig, 2009). An association between aINS activation and subjective experience of anxious anticipation was demonstrated for our auditory anticipation task (Carlson et al., 2011). The dACC, inferior parietal cortex and lPFC mediate attentional control/sustained attention (Bush, Luu, & Posner, 2000; Thakral & Slotnick, 2009). The observed activation in these regions prior to presentation of the affective clips may reflect enhanced vigilance and focus on predictive affect cues. In addition, the dACC is involved in generation of autonomic responses (Critchley et al., 2003). Activity within dACC correlates with measures of skin conductance (Critchley, Mathias, &

Dolan, 2001), blood pressure (Critchley, Corfield, Chandler, Mathias, & Dolan, 2000) and sympathetic cardiovascular influences (Critchley et al., 2003). The thalamus is also involved in the arousal response. Reactivity in the dorsomedial region, in particular, is associated with subjective ratings of arousal (Colibazzi et al., 2010) and intensity of emotional arousal. The caudate (especially the head portion) is often implicated in emotional processing (Lane, 1999). It is part of an output system of affective circuitry involved in motor behaviors (Rolls, 2000) and may contribute to implicit preparatory motor responses to the clips. Additional PFC regions activated during affective anticipation such as the middle and superior frontal gyri have been linked to appraisal processes of expected stimuli and one's reaction to them (Ochsner et al., 2004) as well as automatic (Mauss, Bunge, & Gross, 2007) and volitional emotion regulation (Ochsner et al., 2002; Ochsner & Gross, 2005).

The NAcc and mPFC, which were selectively activated during anticipation of positive clips, are part of the mesocorticolimbic pathways associated with reward and motivation. Both regions are involved in perception of pleasant stimuli (Bartels & Zeki, 2004; Berns, McClure, Pagnoni, & Montague, 2001; Gottfried, O'Doherty, & Dolan, 2002; Karama et al., 2002) and reward processing (S. M. McClure, York, & Montague, 2004). Research on reward anticipation has focused on monetary incentives. This work has implicated the NAcc and mPFC in anticipation of monetary gains (Knutson & Wimmer, 2007) and emphasized their role in assessment of expected value based on gain magnitude and probability (Knutson, Taylor, Kaufman, Peterson, & Glover, 2005). Activity in NAcc prior to delivery of monetary rewards is also associated with self-reported positive arousal (Knutson & Peterson, 2005) and increases in self-reported happiness that may be due in part to dopamine release in this area (Knutson, Adams, Fong, & Hommer, 2001). Anticipatory positive affect has been posited to promote

approach behavior to primary (and secondary) rewards (Knutson & Greer, 2008), which is critical for survival and reproduction.

The amygdala was also active during positive anticipation. Amygdala response to appetitive stimuli (Fitzgerald, Angstadt, Jelsone, Nathan, & Phan, 2006; Hamann & Mao, 2002; O'Doherty, Deichmann, Critchley, & Dolan, 2002) including erotic films (Beauregard, Levesque, & Bourgouin, 2001; Karama et al., 2002) has been previously demonstrated and is in line with a general function in saliency processing that is not valence specific (Liberzon, Phan, Decker, & Taylor, 2003; Sander, Grafman, & Zalla, 2003). During the anticipatory phase the amygdala may additionally facilitate attention and prime visual areas for enhanced processing of the clips (Vuilleumier & Driver, 2007).

A small area in the mid-insula was uniquely activated during anticipation of the aversive clips. Preferential activation in the (anterior) insula has been reported for facial expressions of disgust (Phillips et al., 1997) and disgust-inducing pictures of contamination and mutilation (Wright, He, Shapira, Goodman, & Liu, 2004; but see Phan et al., 2004). In addition, the insula is involved in perception of pain in self and others (i.e., pain empathy; Jackson, Rainville, & Decety, 2006). The aversive clips depicted medical procedures that elicit high levels of disgust (Gross & Levenson, 1995) but due to their graphic nature they may also prompt regions associated with pain processing. The location of insular activation we observed is more compatible with the latter. Interestingly, there was no amygdala activation during aversive anticipation. Amygdala reactivity is less consistently reported for disgusting relative to fearful stimuli (Murphy, Nimmo-Smith, & Lawrence, 2003; Vytal & Hamann, 2010) and is also known to habituate quickly (Breiter et al., 1996). However, it should be noted that the aversive and positive clips were rated as equally arousing and the length of the anticipation period was the

same across conditions

All regions selectively activated during aversive and positive anticipation were also engaged during 'uncertain' anticipation. This finding does not support a negativity bias for anticipated events of unknown valence but rather a preparatory response for both aversive and positive stimuli. The discrepancy with previous results (Herwig et al., 2007) may be due to the use of sexually explicit clips in the current study. These clips are more emotionally intense and have higher motivational value (i.e., motivate approach to a greater extent) than the positive stimuli presented by Herwig and colleagues, which included pictures of animals, scenery and babies. In circumstances when both the aversive and positive stimuli are highly potent, they might be processed equally during the anticipation phase. However, because the aversive stimuli presented by Herwig and colleagues, (e.g., pictures of attacks/mutilations) were potentially more threatening than the disgusting clips used here, our results should be replicated in a paradigm that includes sexual and fear-inducing clips.

Neural response to the affective clips was strong and widespread across temporoparietal visual areas and limbic and paralimbic structures. A comparison of positive and aversive clips showed activity in mesolimbic regions in response to the positive clips and mid-insula activity in response to the aversive clips. This parallels the differential response pattern observed for these two conditions during anticipation. Reactivity to the clips was more extensive than reactivity during the anticipatory phase; however, there was overlap in regions engaged consistent with previous findings that implicate similar networks in both processes (Nitschke et al., 2006). Early recruitment of regions (i.e., during the anticipatory phase) subsequently involved in the response to aversive and other affective stimuli may expedite processing and execution of appropriate action/behavior to these stimuli. Selective activation to the clips was observed in areas involved

in visual processing including subsections of the superior and inferior parietal cortices and fusiform area. In contrast, sections in the right superior frontal gyrus and lPFC, associated with appraisal of stimuli and one's affective state as well as emotion regulation processes, were selectively activated during anticipation.

Self-report anticipatory ratings during the task confirmed that the manipulation was successful in eliciting stronger anticipation to the affective clips compared to the neutral clip. In addition, post-task valence and arousal scores indicate that participants perceived the positive clips as more pleasant than the aversive clips and both as equally arousing. This is important for interpretation of valence effects because it implies that comparisons between the positive and aversive conditions were not confounded by arousal. Pupillary responses, on the other hand, did not differ between the affective and neutral conditions. However, our analysis suggests that this measure may not be optimal for long durations (such as the anticipatory periods used here) due to artifacts.

In summary, our results show that anticipation of affective clips recruits common regions, involved in various preparatory processes, regardless of valence and that additional areas are preferentially engaged depending on whether expected stimuli are negative or positive. Anticipation of clips with 'uncertain' valence reflected a preparatory response for both aversive and positive stimuli. Lastly, a comparison of reactivity during anticipation and clip presentation showed greater engagement of frontal regions during the anticipatory phase. These regions are implicated in appraisal and regulatory processes that may contribute to symptoms of anxiety when maladaptive.

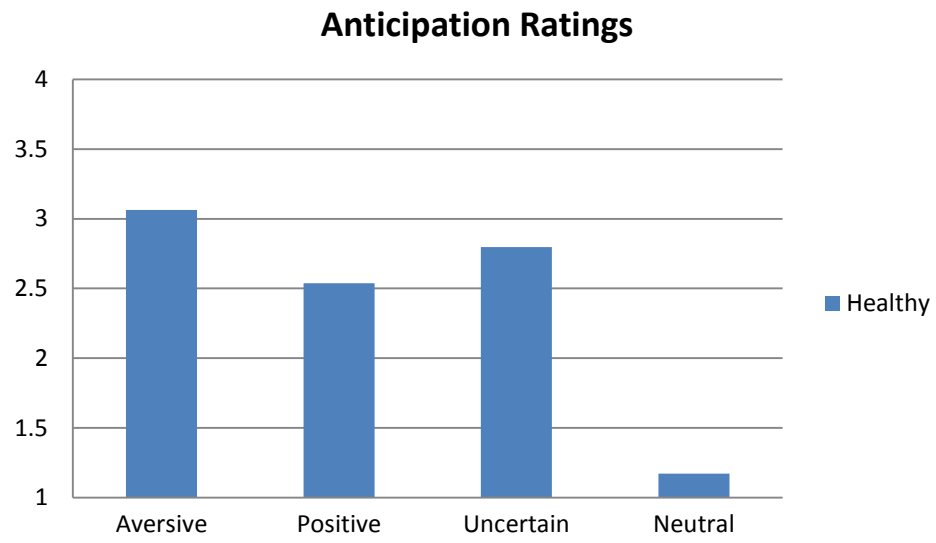
Table 4. Brain activation during aversive, positive, and 'uncertain' anticipation versus neutral anticipation (Healthy group).

Analysis & Region	Hemisphere	MNI Coordinates				Maximum Voxel
		x	*y*	*z*	*voxels*	*T value*
Aversive anticipation > Neutral anticipation						
Anterior insula	R	32	28	-4	387	4.52
	L	-34	24	10	275	4.09
Mid-insula***	R	40	-2	12	12	3.59
Thalamus	R-L	-10	-14	4	1071	4.07
Caudate	R	14	6	14	348	4.08
	L	-14	2	18	364	3.53
Anterior cingulate cortex	R-L	-2	8	28	419	4.57
Superior/Middle frontal gyri***	R	24	58	28		3.49
	L	-46	36	28		3.76
Inferior parietal area (including BA40, BA3)**	R	34	-38	44	75	4.52
	L	-32	-40	38	75	4.56
Positive anticipation > Neutral anticipation						
Anterior Insula	R	30	22	-8	589	4.74
	L	-32	24	0	606	4.65
Thalamus	R-L	-2	-18	10	547	4.34
Caudate	R	-10	6	6	680	6.15
	L	10	8	8	713	5.52
Nucleus accumbens	R	10	8	-4	32	4.24
	L	-12	6	-8	58	5.03
Amygdala	R	18	4	-16	19	3.89
	L	-14	0	-16	75	4.41
Anterior cingulate cortex	R-L	10	20	28	1811	4.81
Medial frontal gyrus*	R-L	4	48	38	140	5.81
Superior/Middle frontal gyri**	R	32	54	22		4.62
	L	-32	56	20		4.46
Inferior parietal area (BA40)**	R	30	-50	60	348	4.33
	L	-38	-40	46	1133	4.53
'Uncertain' anticipation > Neutral anticipation						
Anterior insula	R	32	22	-10	449	4.37
	L	-32	24	-2	762	5.27

Thalamus	R-L	4	-18	4	1177	4.36
Caudate	R	10	10	4	520	4.82
	L	-12	2	12	573	4.86
Nucleus accumbens	L	-12	4	-8	71	4.10
Amygdala	R	18	2	-16	30	3.83
	L	-12	-2	-16	85	4.19
Anterior cingulate cortex	R-L	10	22	28	1203	4.73
Medial frontal gyrus*	R-L	6	44	34	45	5.53
Superior/Middle frontal gyri	R***	28	54	16		3.56
	L**	-36	48	14		4.05
Superior/Middle temporal gyrus**	R	48	-30	4	92	4.30
	L	-52	-28	-4	71	3.99
Inferior parietal area (BA40)**	R	28	-52	58	311	4.93
	L	-34	-48	56	1797	5.73
Midbrain***	R-L	-6	-16	-14		3.89

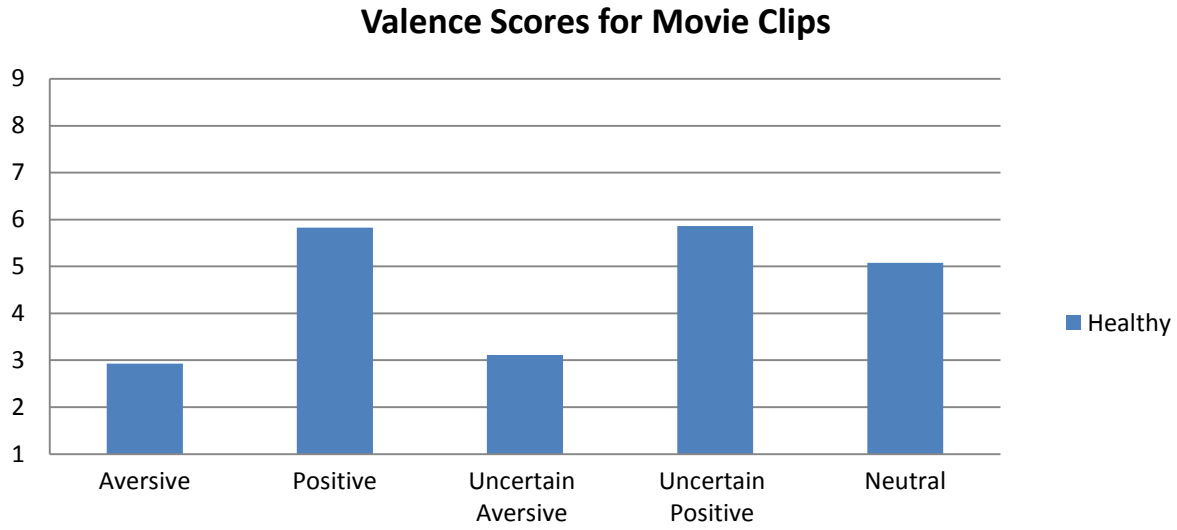
p <.05 small volume correction (SVC); BA = Brodmann area
* p <.05 family-wise error (FWE) corrected for the whole-brain
** p <.0001$_{\text{uncorrected}}$
*** p <.0005$_{\text{uncorrected}}$

Figure 13. Mean anticipatory ratings for the aversive, positive, 'uncertain' and neutral conditions for the Healthy group (N = 24).



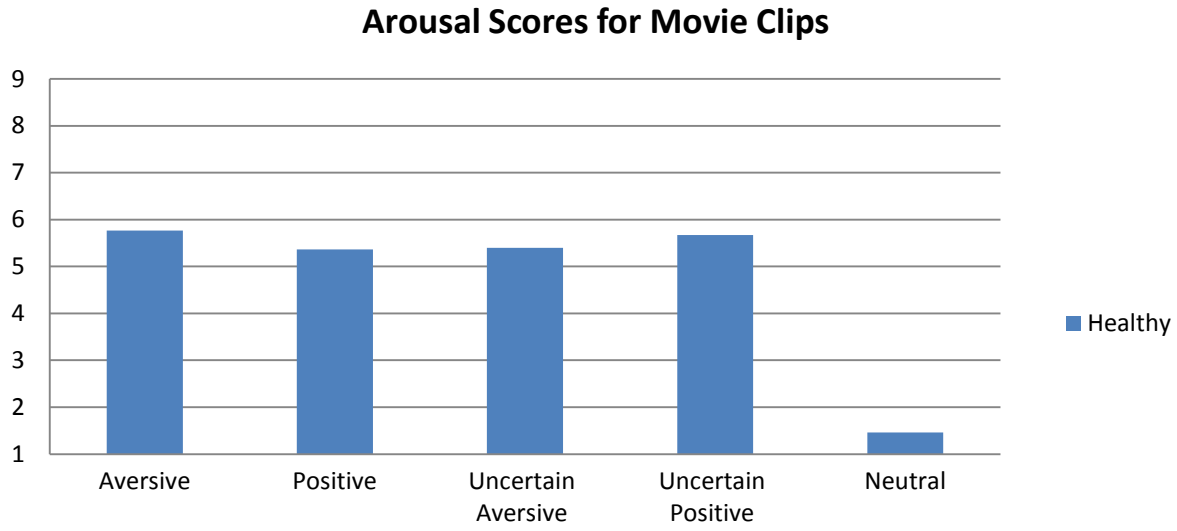
Anticipatory ratings for the aversive, positive and 'uncertain' conditions were all higher than ratings for the neutral condition (all *ps* < .001).

Figure 14. Mean valence scores for the aversive, positive, 'uncertain' aversive, 'uncertain' positive and neutral clips for the Healthy group (N = 23).



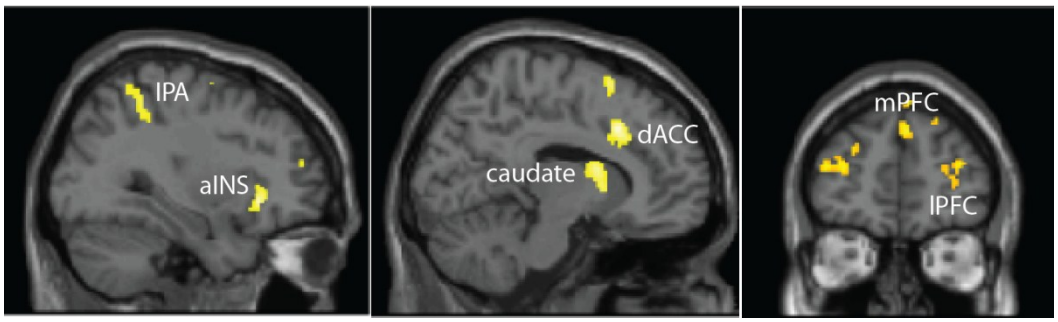
Clips were rated on a 9-point scale ranging from 1 = 'negative' to 9 = 'positive'. Valence scores for the positive and 'uncertain'-positive clips were higher than scores for the aversive and 'uncertain'-aversive clips (*ps* < .001).

Figure 15. Mean arousal scores for the aversive, positive, 'uncertain' aversive, 'uncertain' positive and neutral clips for the Healthy group (N = 23).



Clips were rated on a 9-point scale ranging from 1 = 'calm to 9 = 'excited'. Arousal scores for the aversive, positive, 'uncertain'-aversive and 'uncertain'-positive were higher than scores for the neutral clips (*ps* < .001).

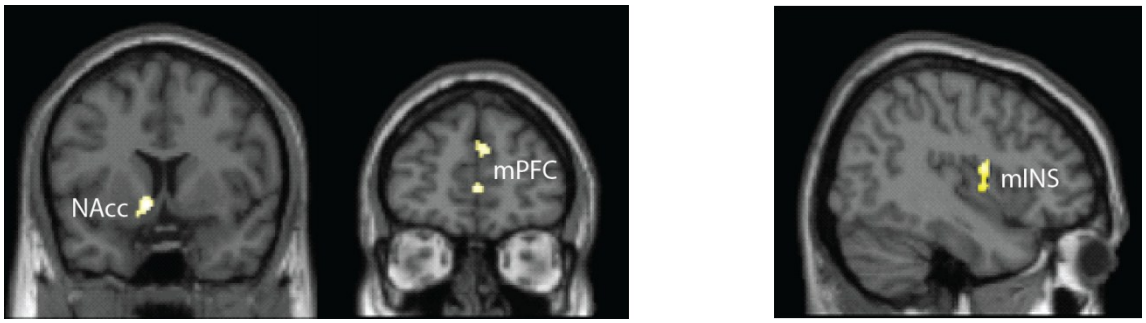
Figure 16. Brain activation for the conjunction of aversive, positive, and 'uncertain' anticipation versus neutral anticipation contrasts (Healthy group).



aINS = anterior insula; **IPA** = inferior parietal area (BA40); **dACC** = dorsal anterior cingulate cortex
mPFC = medial prefrontal cortex; lPFC = lateral prefrontal cortex
Whole brain threshold $\alpha = .001_{uncorrected}$, extent threshold = 10 voxels

Figure 17. Selective activation during positive (left) and aversive (right) anticipation (Healthy group).

(a) Positive anticipation **(b) Aversive anticipation**

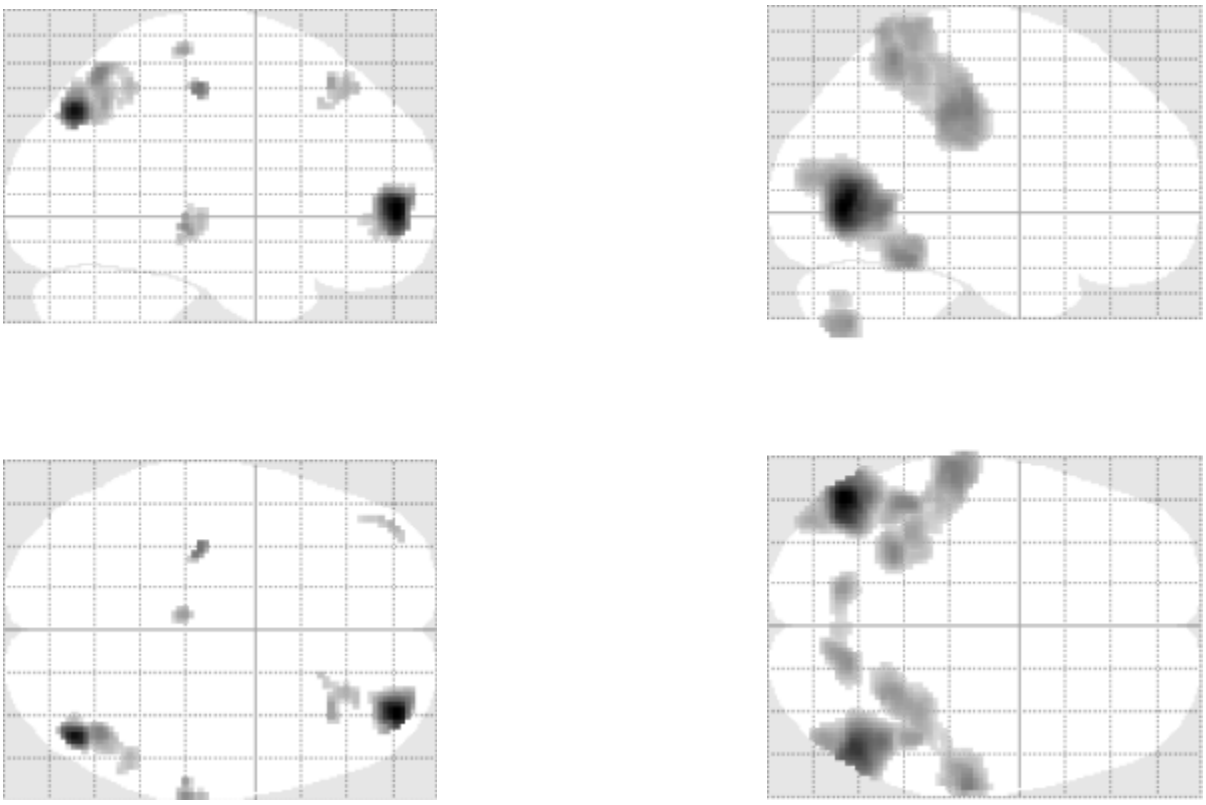


NAcc = nucleus accumbens; **mPFC** = medial prefrontal cortex; **mINS** =mid-insula
Whole brain threshold $\alpha = .001_{uncorrected}$, extent threshold = 10 voxels

Figure 18. Regions preferentially activated during anticipation (left) and clip presentation (right) (Healthy group).

(a) Anticipation > Clip presentation

(b) Clip presentation > Anticipation



Whole brain threshold $\alpha = .001_{uncorrected}$, extent threshold = 10 voxels

Chapter 7: Individuals with Generalized Anxiety Disorder Exhibit Enhanced Anticipatory Response to Neutral Movie Clips

7.1 Introduction

Neuroimaging studies of anticipatory reactivity provide a first step in elucidating the brain regions involved in worry - a defining symptom of generalized anxiety disorder (GAD). These studies have implicated a network of regions that include the insula, anterior cingulate cortex (ACC), amygdala and prefrontal cortex (Nitschke et al., 2006; Simmons et al., 2004).

Anticipatory processing appears to be altered in anxious individuals. High trait-anxious participants show enhanced insula reactivity during anticipation of aversive pictures (Simmons et al., 2006). Furthermore, trait anxiety levels covary with activation in the insula particularly when the anticipated event is near (Carlson et al., 2011). Enhanced insula reactivity has also been observed in clinical populations such as social anxiety disorder (SAD; Lorberbaum et al., 2004; Tillfors, Furmark, Marteinsdottir, & Fredrikson, 2002) and specific phobia (Davidson, Marshall, Tomarken, & Henriques, 2000; Straube et al., 2007) during anticipation of disorder-relevant stimuli. This heightened insula response may reflect greater visceral changes and/or interoceptive awareness during anticipation of potential threat and is consistent with a proposed role for this region in the generation of an interoceptive prediction signal (that represents the difference between current body state and expected aversive body state), which is thought to be augmented in anxious individuals (Paulus & Stein, 2006). The PFC and amygdala also show a differential anticipatory response in anxious individuals; however, findings for these regions have been inconsistent with respect to the direction of activation and specific PFC subregions implicated. For example, Simmons et al. (2006) found lower activation in the medial prefrontal cortex (mPFC) in anxiety-prone individuals, whereas, studies in SAD and specific phobia have reported

enhanced (Straube et al., 2007; Tillfors et al., 2002), reduced (Lorberbaum et al., 2004) and similar (Wik, Fredrikson, & Fischer, 1996) patterns of activation in various prefrontal regions relative to healthy comparisons. As for the amygdala, greater anticipatory reactivity has been observed in SAD (Lorberbaum et al., 2004; Tillfors et al., 2002) but not specific phobia (Wik et al., 1996).

Only one imaging study to date examined anticipatory reactivity in GAD (Nitschke et al., 2009). Patients showed greater activation in the bilateral amygdala relative to healthy controls preceding both aversive and neutral pictures. These findings suggest that individuals with GAD have an indiscriminate anticipatory response that is triggered by a broader range of stimuli including those that are benign. However, it is unknown whether this extends to positive stimuli or situations in which the valence of upcoming events is uncertain. The latter is of particular interest because patients with GAD are thought to be especially averse to uncertainty regarding future outcomes (Dugas, Gagnon, Ladouceur, & Freeston, 1998). Besides the amygdala, there were no group differences in the insula or any other regions associated with anticipation; this discrepancy with findings in other anxious populations requires further investigation as well.

In the current study we used the modified anticipation task to compare reactivity in GAD and healthy individuals across multiple conditions of anticipation: *aversive anticipation, positive anticipation, 'uncertain' anticipation* (in which the probability of seeing an aversive or positive movie clip was unknown to participants) and *neutral anticipation.* Insofar as individuals with GAD have an enhanced and indiscriminate anticipatory response, they would be expected to show greater reactivity for all conditions compared to healthy individuals. Based on evidence for greater intolerance of uncertainty (Ladouceur et al., 1999) and a tendency to interpret neutral/ambiguous stimuli as threatening (Eysenck et al., 1991; Mathews et al., 1989) among

GAD patients, we expected to find greater group differences in anticipatory reactivity for the 'uncertain' and neutral conditions in particular. Anticipatory reactivity was compared between groups for all regions activated in healthy individuals (chapter 6) including the insula, ACC, caudate, nucleus accumbens, thalamus, inferior parietal cortex, PFC and visual areas.

7.2 Method

7.2.1 *Participants*

Thirty-two women with a diagnosis of GAD (*Mean age* = 22.3; *SD* = 4.5) and 25 healthy women (*Mean Age* = 21.6, *SD* = 5.1) were included in the analyses. Participants were screened with the Structured Clinical Interview for DSM-IV Axis I Disorders -Patient Edition, Version 2 (SCID-I/P; First et al., 2002) to confirm diagnoses of GAD in the patient group and absence of current or past Axis I diagnoses in the Healthy group. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

7.2.2 *Image analysis*

Preprocessed images were entered into a general linear model with one regressor for each anticipation period and clip type (aversive, positive, 'uncertain', neutral). In addition, we included the six motion parameters as regressors of no interest. Serial autocorrelations were modeled with an AR (1) process. First-level single subject statistical parameter maps were created for the *aversive anticipation, positive anticipation, 'uncertain' anticipation, neutral anticipation, aversive clip, positive clip, 'uncertain' clip and neutral clip.* These contrasts were entered into a 3 (group: *GAD-only, Comorbid, Healthy*) × 2 (process: *anticipation, clip presentation*) × 4 (valence: *aversive, positive, 'uncertain', neutral*) second-level random effects repeated measures analysis of variance (ANOVA).

7.3 Results

7.3.1 *Self-report anticipatory ratings*

Data for three participants were not available due to technical difficulties. We conducted a 3 (*group: GAD-only, Comorbid, Healthy*) × 4 (*condition: aversive, positive, 'uncertain', neutral*) repeated measures ANOVA to compare anticipatory ratings between groups. Mean scores are presented in figure 19. The main effect of group and group by condition interaction were not significant (both *ps* ≥ .16). However, pairwise comparisons, conducted for exploratory purposes, revealed that individuals in the GAD-only group had a stronger emotional response in anticipation of the neutral clips than individuals in the Healthy (p = .017) or Comorbid (p = .047) groups. Anticipatory ratings did not differ between the Comorbid and Healthy groups for any conditions (all *ps* > .5).

7.3.2 *Post-task valence and arousal ratings of the movie clips*

Ratings for four participants were not available due to software malfunction.

In order to compare valence and arousal scores across groups, we conducted a 3 (*group: GAD-only, Comorbid, Healthy*) × 5 (*condition: aversive, positive, 'uncertain'-aversive, 'uncertain'-positive, neutral*) repeated measures ANOVA for each measure. Mean valence and arousal scores are presented in figures 20 and 21, respectively. There was a significant main effect of group for arousal ratings ($F_{(2,50)}$ = 4.88; p =.012) driven by higher arousal scores in the GAD-only group relative to the Comorbid group (p = .009). Pairwise comparisons between the two groups showed that arousal scores in the GAD-only group were higher for the aversive (p = .034) and positive (p = .032) clips, and marginally higher for the 'uncertain'-aversive clips (p = .07). The main effect of group for valence ratings was not significant (p > .8).

7.3.3 *Between-group comparisons of anticipatory reactivity*

Differences in activation between the GAD group and Healthy group were most pronounced during anticipation of the neutral clips. For this condition, patients showed greater activation in the left insula, caudate, ACC, left amygdala, medial and lateral prefrontal cortex, visual cortex and superior temporal gyrus (see table 6). During aversive, positive and 'uncertain' anticipation, greater activation in the GAD group was observed in primary visual cortex (BA 17) and extrastriate cortex (BA 18 and 19). No brain regions were more activated in the Healthy group for any of these contrasts. In order to test whether additional regions are differentially engaged in the two groups at the beginning of the anticipatory phase, we generated contrasts for the first half (8 seconds) of the anticipation period. This analysis did not provide additional findings. Similar patterns of reactivity were observed for all comparisons when the GAD-only and Comorbid groups were separately compared to the Healthy group. In addition, direct comparisons of the two patient groups did not reveal any differences in reactivity.

7.3.4 *Between-group comparisons of reactivity during presentation of the movie clips*

The GAD group showed greater activation in visual regions, including BA 17 and BA 18 during presentation of the aversive, positive and neutral clips. These differences in visual reactivity were less pronounced than those observed for the two groups during the anticipatory phase.

7.4 Discussion

Patients with generalized anxiety disorder (GAD), compared to healthy individuals, exhibited enhanced reactivity across multiple areas including the left anterior insula (aINS), caudate, left amygdala, anterior cingulate cortex (ACC), middle and inferior frontal gyri, and

temporal and occipital regions during anticipation of the neutral clips, and in visual areas (Brodmann 17, 18 and 19) exclusively, during anticipation of the affective clips. Consistent with a heightened response in the GAD group during neutral anticipation, within-group contrasts of aversive, positive or 'uncertain' anticipation versus neutral anticipation revealed activation in fewer regions compared to similar contrasts in the Healthy group, which commonly engaged the aINS, thalamus, caudate, dorsal ACC, lateral prefrontal cortex (lPFC) and inferior parietal area. Comparisons between the GAD-only and Comorbid groups did not reveal differences in neural reactivity.

Cognitive models of anxiety posit that interpretation of neutral or ambiguous information as threatening contributes to the onset and maintenance of symptoms (Mathews, 1990). This interpretation bias has been demonstrated in GAD using tasks that require participants to write orally presented homophones (e.g., die/dye; Mathews et al., 1989) or complete ambiguous sentences (Eysenck et al., 1991). In addition, GAD patients exhibit negative implicit associations with neutral words related to their worry topic (Reinecke, Becker, Hoyer, & Rinck, 2010) and impaired target detection following neutral pictures in an attentional blink paradigm (Olatunji, Ciesielski, Armstrong, Zhao, & Zald, 2011). Our findings reveal differential processing of neutral stimuli in GAD during the anticipatory phase. Patients showed greater reactivity during anticipation of neutral clips in regions associated with anticipation of threatening (Nitschke et al., 2006) and other emotionally valenced stimuli (Herwig et al., 2007). This is consistent with the notion of an indiscriminate anticipatory response in GAD (Nitschke et al., 2009) and suggests that patients are hypersensitive to anticipation of a broader range of stimuli, even those that are benign. Excessive recruitment of an 'anticipatory network' may contribute to the pervasive nature of worry and anxiety in GAD.

During aversive, positive and 'uncertain' anticipation, the GAD group showed greater reactivity in primary visual (BA 17) and extrastriate cortices (BA 18 and 19). Similar group differences were detected during presentation of the clips but were less pronounced. Activation in visual areas is increased for affective stimuli (Bradley et al., 2003; Lang, Bradley, Fitzsimmons, et al., 1998) likely reflecting adaptive processing of motivationally salient items (Lang, Bradley, & Cuthbert, 1998). This visual response can be modulated by attention (Vuilleumier & Driver, 2007). Several studies have found greater enhancement of visual activation in patients with anxiety relative to healthy comparisons, which may indicate increased vigilance. For example, patients with SAD show increased activation in fusiform gyrus to angry, neutral and happy faces (Straube, Mentzel, & Miltner, 2005) and in occipitotemporal regions (including BA areas 17, 18, 19, 37 and 39) during anticipation of affective pictures (Brühl et al., 2011). Spider phobics show increased activation in fusiform gyrus during anticipation of phobia-related stimuli (Straube et al., 2007). In GAD, present findings reveal an enhanced visual response regardless of stimulus valence or relevance to participants' worry topics suggesting that patients are vigilant to a wide range of stimuli. Indeed, studies using the emotional Stroop and visual probe task that assess attention bias at short durations demonstrate that various stimuli (including disorder-specific, generally aversive and positive) capture patients' attention (Mogg & Bradley, 2005). Visual activation in BA 17 and 18 remained higher in the GAD group during presentation of the clips, which indicates that patients' vigilance during the anticipatory phase does not disengage as previously posited for high trait-anxious individuals (Simmons et al., 2006).

Aside from BA 17 and BA 18, no other brain regions differentiated patients and healthy individuals during anticipation of the affective clips or their presentation. Similar results were

obtained when we compared activation in the two groups during the onset (i.e., first half) of the anticipation period. The type of stimuli used for these conditions, all of which elicited extensive activation in the Healthy group may partly explain the absence of group differences in additional regions associated with anticipation. However, these findings as well as the discrepancy with Nitschke et al's study, in which between-group differences were restricted to the amygdala, require further clarification.

In contrast to the neuroimaging findings, pupil response measures of anticipation did not differ in the GAD and Healthy groups. Future research should investigate this disparity between neural and peripheral physiological responses using alternative measures of anticipation. For the GAD-only and Comorbid groups, there were no differences in neural reactivity, however, scores on self-report measures diverged. Participants in the GAD-only group perceived the affective clips as more arousing and their anticipatory ratings for the neutral condition were marginally higher. To better understand the effect of depressive symptoms on anticipatory processing in GAD it would be useful to compare GAD patients with no comorbidity and patients with major depression disorder (MDD).

In conclusion, our findings provide further evidence for the role of anticipatory processes in the pathophysiology of GAD (Nitschke et al., 2009). Specifically, they suggest that the anticipatory response in GAD patients is triggered by various stimuli including innocuous ones, putting them at risk for constant worry and anxiety.

Table 5. Brain activation during aversive, positive, and 'uncertain' anticipation versus neutral anticipation (GAD group).

Analysis & Region	Hemisphere	MNI Coordinates				Maximum Voxel
		x	*y*	*z*	*voxels*	*T value*
Aversive anticipation >Neutral anticipation						
Anterior insula	R***	36	22	-4	157	3.70
	L	-34	28	6	138	4.66
Inferior parietal area (BA 40)**	R	34	-44	54	48	4.25
	L	-30	-50	56	122	5.11
Positive anticipation > Neutral anticipation						
Anterior insula	R	32	28	4	351	4.89
	L	-32	28	6	415	5.42
Anterior cingulate cortex	R	8	28	28	176	3.62
	L	-4	16	26	162	3.83
Caudate	R	8	8	2	135	5.92
	L	-8	8	-2	76	6.16
Nucleus accumbens	R	8	6	-4	50	5.79
	L	-10	6	-4	37	6.10
Inferior parietal area (BA 40)**	R	34	-40	52	267	5.96
	L	-30	-50	56	175	4.73
'Uncertain' anticipation > Neutral anticipation						
Anterior insula	R	36	22	-4	185	3.87
	L	-32	28	6	163	5.03
Nucleus accumbens	L	-10	-2	-6	143	4.64
Inferior parietal area (BA 40)**	R	34	-38	50	121	4.87
	L	-32	50	58	172	4.75
Midbrain**	R-L	10	-14	-16	17	4.49

$p < .05$ small volume correction (SVC); BA = Brodmann area

**$p < .0001_{\text{uncorrected}}$

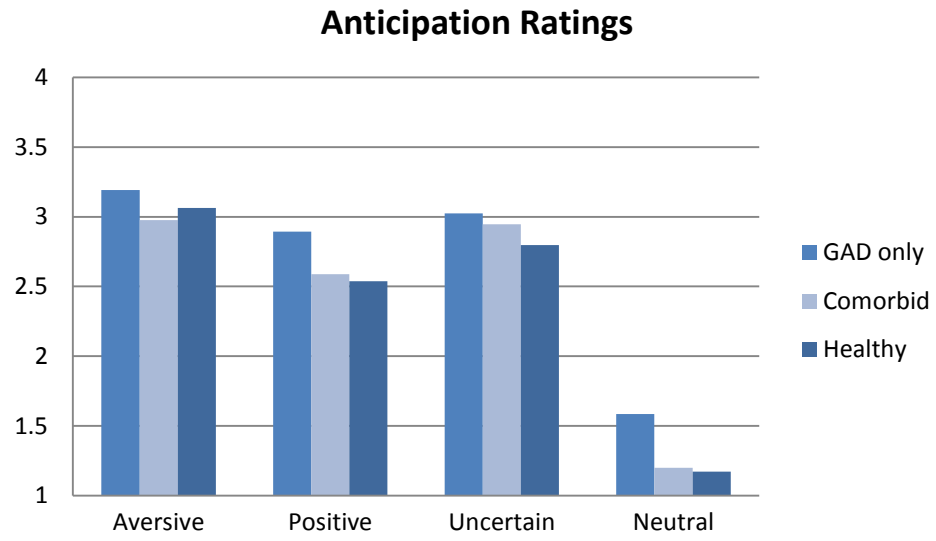
***$p < .0005_{\text{uncorrected}}$

Table 6. Comparison of brain activation for the GAD group (N=32) versus Healthy group (N=25) during neutral anticipation.

Analysis & Region	Hemisphere	MNI Coordinates				Maximum Voxel
		x	*y*	*z*	*voxels*	*T value*
Neutral anticipation (GAD > Healthy)						
Insula	L	-30	20	-20	49	3.88
Caudate	R	14	6	14	331	4.57
	L	-12	0	14	244	4.23
Anterior cingulate cortex	R-L	10	20	26	731	3.95
Amygdala	L	-26	-4	-12	73	4.20
Medial frontal gyrus*	R-L	4	48	40	17	5.03
Middle/Inferior frontal gyri**	R	50	26	28	134	4.60
	L	-42	20	30	439	4.89
Precuneus (BA 7)**	R-L	2	-55	52	80	4.50
Cuneus (BA 17, BA 18)***	R	18	-92	22	394	4.37
	L	-12	-92	0	126	4.08
Inferior parietal area (BA40)***	R	42	-56	44	51	3.59
	L	-50	-44	46	368	3.95
Superior/Middle temporal gyrus**	R	48	-24	4	25	4.33
	L	-58	-28	0	76	4.95

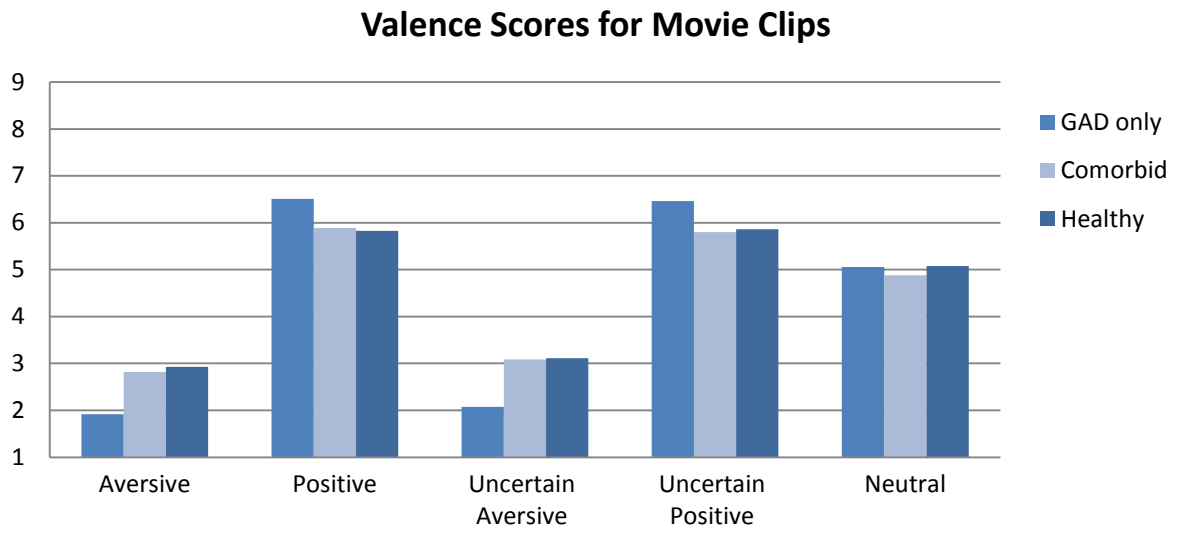
$p < .05$ small volume correction (SVC); BA = Brodmann area
* $p < .05$ family-wise error (FWE) corrected for the whole-brain
** $p < .0001_{\text{uncorrected}}$
*** $p < .0005_{\text{uncorrected}}$

Figure 19. Mean anticipatory ratings for the aversive, positive, 'uncertain' and neutral conditions for the GAD-only, Comorbid and Healthy groups (Total N = 54).



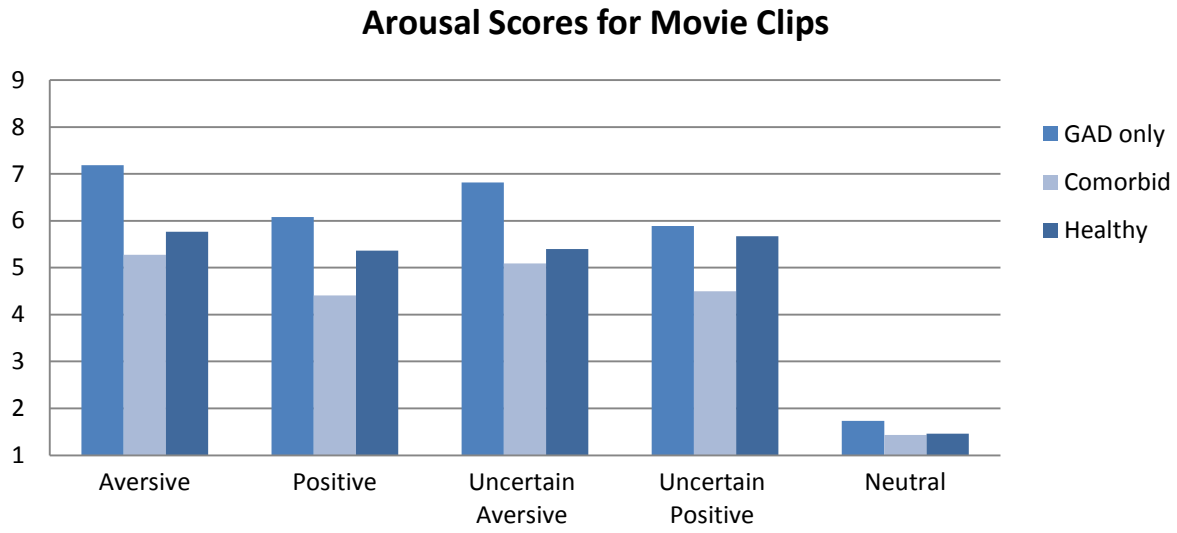
Individuals in the GAD-only group had a stronger emotional response in anticipation of the neutral clips than individuals in the Healthy (p = .017) and Comorbid (p = .047) groups (Bonferroni adjusted p values).

Figure 20. Mean valence scores for the aversive, positive, 'uncertain' aversive, 'uncertain' positive and neutral clips for the GAD-only, Comorbid and Healthy groups (N = 54)



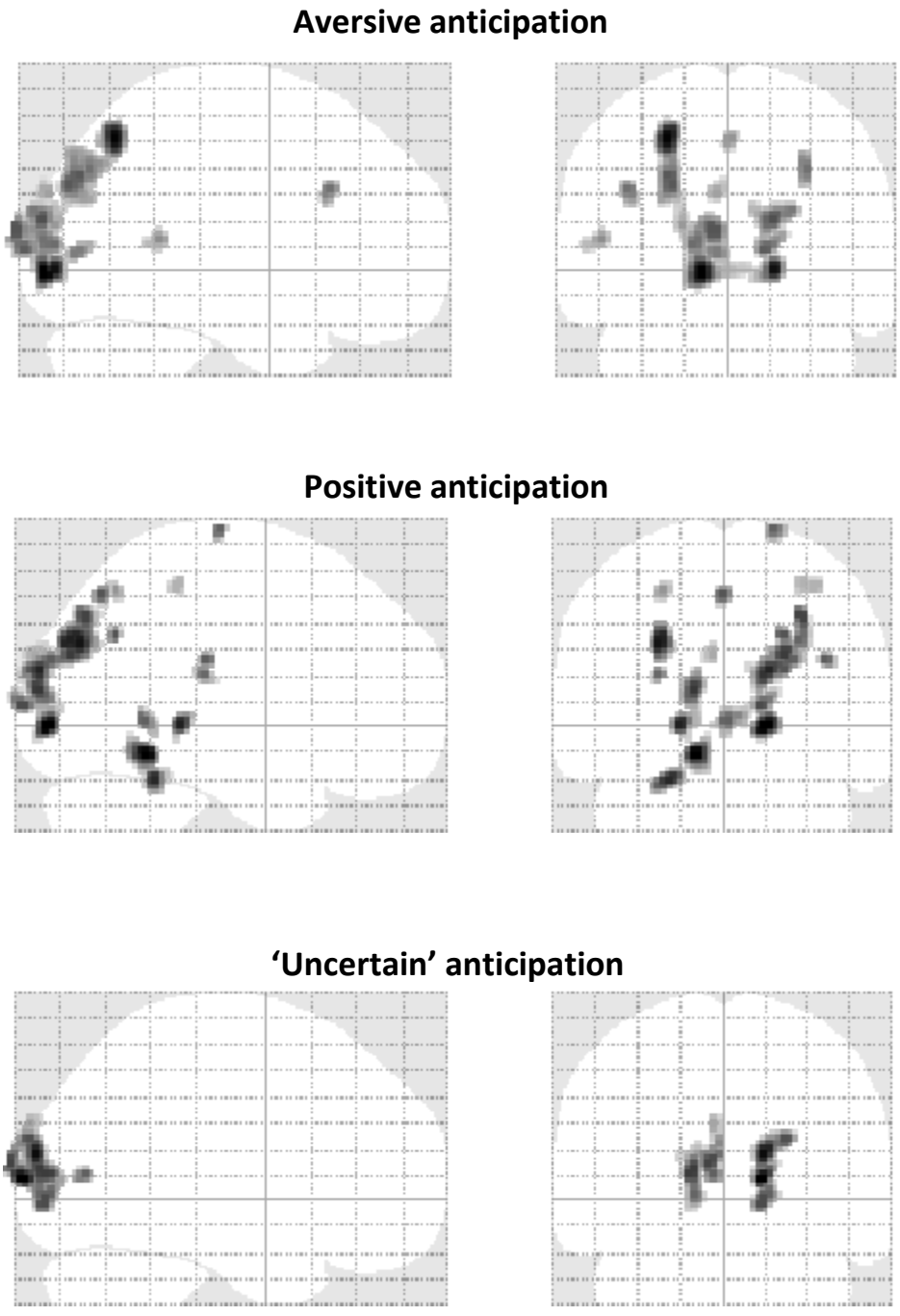
Clips were rated on a 9-point scale ranging from 1 = 'negative' to 9 = 'positive'.

Figure 21. Mean arousal scores for the aversive, positive, 'uncertain' aversive, 'uncertain' positive and neutral clips for the GAD-only, Comorbid and Healthy groups (N = 53).



Clips were rated on a 9-point scale ranging from 1 = 'calm to 9 = 'excited'. Arousal scores in the GAD-only group were higher, compared to the Comorbid group, for the aversive (p = .034) and positive (p = .032) clips and marginally higher for the 'uncertain'-aversive clips (p = .07; Bonferroni adjusted p values).

Figure 22. Comparisons of brain activation for the GAD group (N=32) versus Healthy group (N=25) during aversive, positive, and 'uncertain' anticipation show greater activation in visual areas in GAD.



Whole brain threshold $\alpha = .001_{uncorrected}$, extent threshold = 10 voxels

Chapter 8: General Discussion

The current study investigated the neural basis of generalized anxiety disorder (GAD) – a prevalent yet understudied disorder – using two paradigms: *fear generalization* and *anticipation*. Recent studies on fear generalization have demonstrated that fear-potentiated startle and skin conductance responses to a conditioned stimulus (CS) generalize to similar stimuli, with the strength of the fear response linked to perceptual similarity to the CS (Hajcak et al., 2009; Lissek et al., 2008). Importantly, there is evidence that this fear gradient is flatter in clinically anxious individuals, reflecting greater generalization (Lissek et al., 2010). In this study, we sought to extend previous research by elucidating the brain regions involved in fear generalization and examining whether reactivity in these regions differs in patients with GAD compared to healthy individuals. Anticipation studies have helped to identify a network of regions involved in preparatory responses to affective stimuli. Preliminary findings suggest that GAD patients have a less discriminate anticipatory response, compared to healthy individuals, as evidenced by greater amygdala activation preceding both aversive and neutral stimuli, (Nitschke et al., 2009). Here, we employed a modified anticipation task with multiple conditions and high arousal stimuli to examine the effects of valence and uncertainty on the anticipatory response in GAD and to assess its discriminability.

Results for the generalization task show that neural reactivity in several brain regions that mediate the fear response including the insula, anterior cingulate cortex (ACC), supplementary motor area (SMA) and caudate track generalization gradients of conditioned fear. Across participants, reactivity in these regions was characterized by a peak response to the CS that declines with greater perceptual dissimilarity of the generalization stimuli (GS) to the CS. A similar gradient response pattern was observed for self-report and autonomic measures

associated with these stimuli. Reactivity in the ventromedial prefrontal cortex (vmPFC), a region involved in fear inhibition, showed an opposite response pattern (i.e., largest response to the GS most dissimilar to the CS). In GAD, the vmPFC gradient was characterized by a flat slope with gradual increases in reactivity as GS became more dissimilar to the CS. This vmPFC gradient type was associated with higher levels of anxiety and depressive symptoms in patients. Healthy individuals, on the other hand, exhibited a steeper vmPFC gradient slope with greater increases in vmPFC reactivity to consecutive GS. Flatter vmPFC gradients in GAD suggest that recruitment of this region may be deficient – impeding inhibition of fear responses – during exposure to stimuli that are similar to danger cues, which could facilitate fear generalization. Enhanced generalization in GAD could contribute to the maintenance and /or exacerbation of symptoms by increasing the number of cues/events capable of triggering worry behavior and anxiety. This process can occur based on various shared features across stimuli beyond perceptual similarity and hence, potentially widespread. It is unclear from our results whether the altered vmPFC response in the GAD group is related to impairments in discriminative conditioning. Neural reactivity in regions that mediate the fear response as well as autonomic responses to the CS and GS did not differ in patients and healthy individuals; nor did reactivity in the hippocampus – a region that is thought to mediate stimulus representation (Gluck, Myers, & Meeter, 2005). However, less discriminant reactivity in somatosensory cortex across stimuli and greater variability in post-task ratings of shock likelihood in the GAD group point to possible deficits in internalizing stimulus-shock contingencies.

During anticipation of all affective clips a set of common regions consisting of the anterior insula (aINS), thalamus, dorsal anterior cingulate cortex (dACC), prefrontal cortex and inferior parietal cortex were engaged; these regions are involved in various preparatory processes

such as vigilance, increased arousal, appraisal, and emotion regulation. The nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC) – regions implicated in reward processing – were selectively activated during anticipation of positive clips and the mid-insula, a region involved in pain processing, was selectively activated during aversive anticipation. Thus, anticipation of affective clips recruits a shared circuitry involved in various preparatory processes regardless of valence with additional regions selectively engaged depending on whether expected stimuli are positive or negative. The insula, ACC and PFC are also implicated in anticipation of auditory and tactile stimuli suggesting that a core circuitry of anticipation is recruited across modalities (Carlson et al., 2011; Chua et al., 1999; Drabant et al., 2011). Selective engagement of the NAcc and mid-insula may reflect an adaptive mechanism promoting approach and avoidance behavior based on stimulus valence.

Patients with GAD, compared to healthy individuals, showed enhanced reactivity during neutral anticipation in multiple areas including the left anterior insula (aINS), ACC, posterior cingulate cortex (PCC), caudate, thalamus, left amygdala, PFC, temporal and occipital regions. This is consistent with an indiscriminate anticipatory response in GAD (Nitschke et al., 2009) and suggests that patients are hypersensitive to anticipation of any stimuli, even those that are benign. During anticipation of affective clips, patients exhibited a selective increase in visual cortical activation. Enhancement in visual reactivity to affective stimuli has been previously observed in other anxious populations (Straube et al., 2005, 2007) and likely reflects increased vigilance during both expectation and actual exposure to emotional stimuli. The absence of group differences in additional regions associated with anticipation may be partly explained by the high intensity of the aversive and positive movie clips, which elicited strong activation in the Healthy group.

Findings from the generalization and anticipation tasks underscore altered processing of innocuous stimuli in GAD – both in terms of regulation of fear responses associated with such stimuli and enhanced reactivity during their anticipation. In line with current results, deficient vmPFC response in GAD has been observed during implicit regulation of emotional conflict (Etkin et al., 2010). Furthermore, a recent study found that patients exhibit reduced structural integrity in bilateral uncinate fasciculus – a frontolimbic white matter tract (Tromp et al., 2012); reductions in structural connectivity were associated with more negative functional coupling between the pregenual ACC and the amygdala during aversive anticipation. Taken together, these findings provide evidence that deficits in PFC and its connectivity are associated with impaired regulatory skills in GAD consistent with patients' reported difficulties in controlling their worry and anxiety. A number of studies have demonstrated a tendency in GAD patients to attend to (Olatunji et al., 2011) and interpret (Eysenck et al., 1991; Mathews et al., 1989) neutral/ambiguous stimuli as threatening. This processing bias can contribute to the pervasiveness of symptoms by increasing the likelihood that patients will identify threat cues. Excessive recruitment of an 'anticipatory network' that includes the insula, ACC, amygdala and PFC preceding innocuous stimuli provides neurobiological support for such an effect.

PFC responses in the GAD group showed opposite patterns for the two tasks. Patients, compared to healthy individuals, exhibited diminished vmPFC reactivity during presentation of the GS and increased lPFC and mPFC reactivity during neutral anticipation. These opposing reactivity patterns may relate to differences in the regulatory mechanisms involved in the two tasks. More specifically, vmPFC hypoactivation during the short periods of GS presentation may reflect deficits in implicit (automatic) regulation of fear, whereas hyperactivation in lPFC and mPFC during the long anticipatory periods may reflect increased worry or compensatory

activation associated with uninstructed explicit (volitional) emotion regulation. This alludes to a more intricate role for the PFC in the psychopathology of GAD that could account for reports of diminished and enhanced PFC reactivity in previous studies.

Cognitive behavioral therapy (CBT) is commonly used to treat GAD (Hunot, Churchill, Silva de Lima, & Teixeira, 2007). Based on current findings, certain procedures employed within CBT may be especially beneficial for patients. For example, self-monitoring could improve patients' ability to discriminate safe and unsafe cues and promote recognition of common triggers of worry and anxiety; this would facilitate a sense of control and allow early employment of adaptive responses. Interpretation modification techniques can be used to reinforce nonthreatening interpretations of neutral/ambiguous information. Patients would also benefit from emotion regulation training aimed at developing appropriate and flexible regulatory skills. Through repetitive training of explicit regulation strategies, implicit regulation processes could be modified as well (Gyurak, Gross, & Etkin, 2011). Deficits in fear inhibition may also be targeted pharmacologically with d-cycloserine. This antibiotic enhances extinction learning in rats (Walker, Ressler, Lu, & Davis, 2002) and has been shown to augment exposure therapy in social anxiety disorder (Hofmann et al., 2006) and specific phobia (Ressler et al., 2004) but has yet to be tested in GAD. The efficacy of these procedures could be assessed with neuroimaging before and after treatment with focus on changes in PFC response. A preliminary investigation showed increases in vlPFC activation following successful treatment with CBT or fluoxetine in adolescents with GAD (Maslowsky et al., 2010). Furthermore, heightened pregenual ACC reactivity in GAD patients prior to treatment, which may reflect preserved regulatory skills, was associated with greater reductions in anxiety and worry symptoms following treatment with Venlafaxine (a serotonin-norepinephrine reuptake inhibitor; Nitschke et al., 2009).

The current study provides the first attempt to examine neural reactivity associated with fear generalization in clinical anxiety. Findings extend previous research by identifying a circuitry involved in this important learning mechanism and revealing a distinctive vmPFC response pattern in GAD suggestive of deficits in fear regulation. We introduce a novel method of quantifying and presenting brain activation associated with fear generalization – neural gradients – that may serve as potential markers for diagnosis and/or prediction of treatment response together with other psychophysiological gradients. Findings from the modified anticipation task add to the literature by demonstrating differential recruitment of brain regions during anticipation of high arousal aversive and positive stimuli. Moreover, they provide support for a heightened and indiscriminate anticipatory response in GAD within a paradigm that allowed more exhaustive examination of anticipatory processes and a larger sample, compared to previous studies.

The generalization task utilized GS that varied along a physically neutral stimulus dimension (i.e., size). Future studies will examine whether changes in fear-relevant attributes of the GS result in greater increases of fear generalization in anxious individuals. These types of stimuli will be easier to test in phobia and post-traumatic stress disorder (PTSD) where the fear response is triggered by more specific objects/events compared to GAD in which worry behavior encompasses multiple domains and anxiety is more diffused. In addition, it will be useful to collect imaging data during the conditioning phase to assess group differences in fear acquisition and their effect on generalization; time constraints precluded collection of such data for this study. Finally, functional interactions among brain regions associated with generalization need to be further explored, in particular, inputs to PFC from regions such as the amygdala, hippocampus and striatum, which provide information regarding stimulus predictive value and

context, as these may affect vmPFC response during fear inhibition. With respect to the anticipation task, the role of brain regions involved in the various preparatory processes that occur during anticipation should be better specified. For example, incorporating conditions of instructed emotion regulation and instructed worry in future paradigms could aid interpretation of frontal activation. These conditions would also be instrumental for investigating the opposing PFC responses in GAD and their relation to deficits in regulatory processes. Studies should also establish a better physiological/autonomic correlate of anticipatory anxiety in GAD. The pupillary response measure, employed in this study, was vulnerable to artifacts when acquired for long periods and therefore is not optimal for quantifying arousal during the anticipation phase. A potential alternative is to use surface electromyography (EMG) to assess muscle tension – the most common somatic symptom reported by GAD patients (Huppert & Sanderson, 2010). The impact of comorbid depression in GAD also requires clarification. While there were no differences between the GAD and Comorbid groups in neural and autonomic reactivity, self-report ratings point to group variations in anticipatory anxiety and perception of affective stimuli. Inclusion of a third comparison group comprised of MDD patients would help to distinguish neuroanatomical and functional correlates associated with anxiety and depressive symptoms. In addition, future studies should investigate whether GAD patients with longer illness durations present with additional alterations in neural reactivity during fear generalization and anticipation as well as examine male participants. Finally, findings in GAD should be extended to other anxiety disorders to assess generalization and anticipatory processes in relation to different anxiety symptoms.

References

Abel, J. L., & Borkovec, T. D. (1995). Generalizability of DSM-III—R generalized anxiety disorders to proposed DSM-IV criteria and cross-validation of proposed changes. *Journal Of Anxiety Disorders, 9*(4), 303-315. doi: 10.1016/0887-6185(95)00011-c

American Psychiatric Association. (1987). *Diagnostic and statistical manual of mental disorders (3rd ed, rev)*. Washington, DC: Author.

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders (4th ed.)*. Washington, DC: Author.

Antony, M. M., Orsillo, S. M., & Roemer, L. (2001). *Practitioner's guide to empirically based measures of anxiety*. Dordrecht Netherlands: Kluwer Academic Publishers.

Augustine, J. R. (1996). Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Res Brain Res Rev, 22*(3), 229-244.

Bach, D. R., Weiskopf, N., & Dolan, R. J. (2011). A stable sparse fear memory trace in human amygdala. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 31*(25), 9383-9389. doi: 10.1523/JNEUROSCI.1524-11.2011

Barlow, D. H. (2000). Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory. *American Psychologist, 55*(11), 1247-1263. doi: 10.1037/0003-066x.55.11.1247

Barnes, L. L. B., Harp, D., & Jung, W. S. (2002). Reliability generalization of scores on the Spielberger State-Trait Anxiety Inventory. *Educational and Psychological Measurement, 62*(4), 603-618. doi: 10.1177/0013164402062004005

Bartels, A., & Zeki, S. (2004). The neural correlates of maternal and romantic love. *NeuroImage, 21*(3), 1155-1166. doi: 10.1016/j.neuroimage.2003.11.003

Beauregard, M., Levesque, J., & Bourgouin, P. (2001). Neural correlates of conscious self-regulation of emotion. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 21*(18), RC165.

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation.

Berns, G. S., McClure, S. M., Pagnoni, G., & Montague, P. R. (2001). Predictability modulates human brain response to reward. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 21*(8), 2793-2798.

Blair, K., Shaywitz, J., Smith, B. W., Rhodes, R., Geraci, M., Jones, M., . . . Pine, D. S. (2008). Response to emotional expressions in generalized social phobia and generalized anxiety disorder: evidence for separate disorders. *Am J Psychiatry, 165*(9), 1193-1202. doi: 10.1176/appi.ajp.2008.07071060

Borkovec, T. D. (1994). The nature, functions, and origins of worry. In G. C. L. Davey & F. Tallis (Eds.), *Worrying: Perspectives on theory, assessment and treatment.* (pp. 5-33). Oxford England: John Wiley & Sons.

Borkovec, T. D., Shadick, R. N., & Hopkins, M. (1991). The nature of normal and pathological worry. In R. M. Rapee & D. H. Barlow (Eds.), *Chronic anxiety: Generalized anxiety disorder and mixed anxiety-depression.* (pp. 29-51). New York, NY US: Guilford Press.

Bradley, M. M., Sabatinelli, D., Lang, P. J., Fitzsimmons, J. R., King, W., & Desai, P. (2003). Activation of the visual cortex in motivated attention. *Behavioral neuroscience, 117*(2), 369-380.

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., . . . Rosen, B. R. (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron, 17*(5), 875-887.

Bremner, J. D., Vermetten, E., Schmahl, C., Vaccarino, V., Vythilingam, M., Afzal, N., . . . Charney, D. S. (2005). Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychol Med, 35*(6), 791-806.

Brown, T. A., O'Leary, T. A., & Barlow, D. H. (2001). Generalized anxiety disorder. In D. H. Barlow (Ed.), *Clinical handbook of psychological disorders: A step-by-step treatment manual (3rd ed.).* (pp. 154-208). New York, NY US: Guilford Press.

Brühl, A. B., Rufer, M., Delsignore, A., Kaffenberger, T., Jäncke, L., & Herwig, U. (2011). Neural correlates of altered general emotion processing in social anxiety disorder. *Brain Research, 1378*, 72-83. doi: 10.1016/j.brainres.2010.12.084

Büchel, C., & Dolan, R. J. (2000). Classical fear conditioning in functional neuroimaging. *Current Opinion in Neurobiology, 10*(2), 219-223. doi: 10.1016/s0959-4388(00)00078-7

Büchel, C., Morris, J., Dolan, R. J., & Friston, K. J. (1998). Brain systems mediating aversive conditioning: an event-related fMRI study. *Neuron, 20*(5), 947-957.

Bush, G., Luu, P., & Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci, 4*(6), 215-222.

Carlson, J. M., Greenberg, T., Rubin, D., & Mujica-Parodi, L. R. (2011). Feeling anxious: anticipatory amygdalo-insular response predicts the feeling of anxious anticipation. *Social Cognitive And Affective Neuroscience, 6*(1), 74-81. doi: 10.1093/scan/nsq017

Carlson, J. M., & Mujica-Parodi, L. R. (2010). A disposition to reappraise decreases anterior insula reactivity during anxious anticipation. *Biol Psychol, 85*(3), 383-385. doi: 10.1016/j.biopsycho.2010.08.010

Chua, P., Krams, M., Toni, I., Passingham, R., & Dolan, R. (1999). A functional anatomy of anticipatory anxiety. *NeuroImage, 9*(6 Pt 1), 563-571. doi: 10.1006/nimg.1999.0407

Ciocchi, S., Herry, C., Grenier, F., Wolff, S. B., Letzkus, J. J., Vlachos, I., . . . Luthi, A. (2010). Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature, 468*(7321), 277-282. doi: 10.1038/nature09559

Colibazzi, T., Posner, J., Wang, Z., Gorman, D., Gerber, A., Yu, S., . . . Peterson, B. S. (2010). Neural systems subserving valence and arousal during the experience of induced emotions. *Emotion, 10*(3), 377-389. doi: 10.1037/a0018484

Corcoran, K. A., & Quirk, G. J. (2007). Recalling safety: Cooperative functions of the ventromedial prefrontal cortex and the hippocampus in extinction. *CNS Spectrums, 12*(3), 200-206.

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews. Neuroscience, 3*(8), 655-666.

Craig, A. D. (2009). How do you feel--now? The anterior insula and human awareness. *Nature Reviews. Neuroscience, 10*(1), 59-70.

Critchley, H. D., Corfield, D. R., Chandler, M. P., Mathias, C. J., & Dolan, R. J. (2000). Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. *The Journal Of Physiology, 523 Pt 1*, 259-270.

Critchley, H. D., Mathias, C. J., & Dolan, R. J. (2001). Neural activity in the human brain relating to uncertainty and arousal during anticipation. *Neuron, 29*(2), 537-545.

Critchley, H. D., Mathias, C. J., Josephs, O., O'Doherty, J., Zanini, S., Dewar, B. K., . . . Dolan, R. J. (2003). Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain : a journal of neurology, 126*(Pt 10), 2139-2152. doi: 10.1093/brain/awg216

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., & Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nature Neuroscience, 7*(2), 189-195. doi: 10.1038/nn1176

Davidson, R. J., Marshall, J. R., Tomarken, A. J., & Henriques, J. B. (2000). While a phobic waits: regional brain electrical and autonomic activity in social phobics during anticipation of public speaking. *Biol Psychiatry, 47*(2), 85-95.

Davis, M. (1992). The role of the amygdala in conditioned fear. In J. P. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction.* (pp. 255-306). New York, NY US: Wiley-Liss.

Delgado, M. R., Li, J., Schiller, D., & Phelps, E. A. (2008). The role of the striatum in aversive learning and aversive prediction errors. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 363*(1511), 3787-3800. doi: 10.1098/rstb.2008.0161

Delgado, M. R., Nearing, K. I., Ledoux, J. E., & Phelps, E. A. (2008). Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron, 59*(5), 829-838. doi: 10.1016/j.neuron.2008.06.029

Delgado, M. R., Olsson, A., & Phelps, E. A. (2006). Extending animal models of fear conditioning to humans. *Biological Psychology, 73*(1), 39-48. doi: 10.1016/j.biopsycho.2006.01.006

Drabant, E. M., Kuo, J. R., Ramel, W., Blechert, J., Edge, M. D., Cooper, J. R., . . . Gross, J. J. (2011). Experiential, autonomic, and neural responses during threat anticipation vary as a function of threat intensity and neuroticism. *NeuroImage, 55*(1), 401-410.

Dugas, M. J., Anderson, K. G., Deschenes, S. S., & Donegan, E. (2010). Generalized anxiety disorder publications: where do we stand a decade later? *Journal Of Anxiety Disorders, 24*(7), 780-784.

Dugas, M. J., Freeston, M. H., & Ladouceur, R. (1997). Intolerance of uncertainty and problem orientation in worry. *Cognitive Therapy and Research, 21*(6), 593-606. doi: 10.1023/a:1021890322153

Dugas, M. J., Gagnon, F., Ladouceur, R., & Freeston, M. H. (1998). Generalized anxiety disorder: a preliminary test of a conceptual model. *Behav Res Ther, 36*(2), 215-226.

Dugas, M. J., Marchand, A., & Ladouceur, R. (2005). Further validation of a cognitive-behavioral model of generalized anxiety disorder: Diagnostic and symptom specificity. *Journal Of Anxiety Disorders, 19*(3), 329-343. doi: 10.1016/j.janxdis.2004.02.002

Dunsmoor, J. E., Mitroff, S. R., & LaBar, K. S. (2009). Generalization of conditioned fear along a dimension of increasing fear intensity. *Learn Mem, 16*(7), 460-469. doi: 10.1101/lm.1431609

Dunsmoor, J. E., Prince, S. E., Murty, V. P., Kragel, P. A., & LaBar, K. S. (2011). Neurobehavioral mechanisms of human fear generalization. *NeuroImage, 55*(4), 1878-1888. doi: 10.1016/j.neuroimage.2011.01.041

Dunsmoor, J. E., White, A. J., & LaBar, K. S. (2011). Conceptual similarity promotes generalization of higher order fear learning. *Learn Mem, 18*(3), 156-160. doi: 10.1101/lm.2016411

Etkin, A., Prater, K. E., Hoeft, F., Menon, V., & Schatzberg, A. F. (2010). Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *Am J Psychiatry, 167*(5), 545-554. doi: 10.1176/appi.ajp.2009.09070931

Etkin, A., Prater, K. E., Schatzberg, A. F., Menon, V., & Greicius, M. D. (2009). Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Archives of general psychiatry, 66*(12), 1361-1372. doi: 10.1001/archgenpsychiatry.2009.104

Etkin, A., & Wager, T. D. (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry, 164*(10), 1476-1488. doi: 10.1176/appi.ajp.2007.07030504

Eysenck, M. W., Mogg, K., May, J., Richards, A., & Mathews, A. (1991). Bias in interpretation of ambiguous sentences related to threat in anxiety. *J Abnorm Psychol, 100*(2), 144-150.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition.(SCID-I/P)* New York: Biometrics Research, New York State Psychiatric Institute.

Fitzgerald, D. A., Angstadt, M., Jelsone, L. M., Nathan, P. J., & Phan, K. L. (2006). Beyond threat: amygdala reactivity across multiple expressions of facial affect. *NeuroImage, 30*(4), 1441-1448. doi: 10.1016/j.neuroimage.2005.11.003

Freeston, M. H., Rhéaume, J., Letarte, H., & Dugas, M. J. (1994). Why do people worry? *Personality and Individual Differences, 17*(6), 791-802. doi: 10.1016/0191-8869(94)90048-5

Friston, K. J., Buechel, C., Fink, G. R., Morris, J., Rolls, E., & Dolan, R. J. (1997). Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage, 6*(3), 218-229. doi: 10.1006/nimg.1997.0291

Gluck, M. A., & Myers, C. E. (1993). Hippocampal mediation of stimulus representation: a computational theory. *Hippocampus, 3*(4), 491-516.

Gluck, M. A., Myers, C. E., & Meeter, M. (2005). Cortico-hippocampal interaction and adaptive stimulus representation: A neurocomputational theory of associative learning and memory. *Neural Networks, 18*(9), 1265-1279. doi: 10.1016/j.neunet.2005.08.003

Goisman, R. M., Goldenberg, I., Vasile, R. G., & Keller, M. B. (1995). Comorbidity of anxiety disorders in a multicenter anxiety study. *Compr Psychiatry, 36*(4), 303-311.

Gottfried, J. A., & Dolan, R. J. (2004). Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nature Neuroscience, 7*(10), 1144-1152.

Gottfried, J. A., O'Doherty, J., & Dolan, R. J. (2002). Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 22*(24), 10829-10837.

Greenberg, T., Carlson, J. M., Cha, J., Hajcak, G., & Mujica-Parodi, L. R. (2011). Neural reactivity tracks fear generalization gradients. *Biol Psychol*. doi: 10.1016/j.biopsycho.2011.12.007

Gross, J. J., & Levenson, R. W. (1995). Emotion elicitation using films. *Cognition & Emotion, 9*(1), 87-108. doi: 10.1080/02699939508408966

Gyurak, A., Gross, J. J., & Etkin, A. (2011). Explicit and implicit emotion regulation: a dual-process framework. *Cogn Emot, 25*(3), 400-412. doi: 10.1080/02699931.2010.544160

Hajcak, G., Castille, C., Olvet, D. M., Roohi, J., Hatchwell, E., & Dunning, J. P. (2009). Genetic variation in brain-derived neurotrophic factor and human fear conditioning. *Genes, Brain & Behavior, 8*(1), 80-85. doi: 10.1111/j.1601-183X.2008.00447.x

Hamann, S., & Mao, H. (2002). Positive and negative emotional verbal stimuli elicit activity in the left amygdala. *Neuroreport, 13*(1), 15-19. doi: 10.1097/00001756-200201210-00008

Herwig, U., Kaffenberger, T., Baumgartner, T., & Jäncke, L. (2007). Neural correlates of a 'pessimistic' attitude when anticipating events of unknown emotional valence. *NeuroImage, 34*(2), 848-858.

Hoffman, D. L., Dukes, E. M., & Wittchen, H. U. (2008). Human and economic burden of generalized anxiety disorder. *Depress Anxiety, 25*(1), 72-90. doi: 10.1002/da.20257

Hofmann, S. G., Meuret, A. E., Smits, J. A., Simon, N. M., Pollack, M. H., Eisenmenger, K., . . . Otto, M. W. (2006). Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. *Archives of general psychiatry, 63*(3), 298-304. doi: 10.1001/archpsyc.63.3.298

Hunot, V., Churchill, R., Silva de Lima, M., & Teixeira, V. (2007). Psychological therapies for generalised anxiety disorder. *Cochrane Database Of Systematic Reviews (Online)*(1), CD001848.

Hupé, J.-M., Lamirel, C., & Lorenceau, J. (2009). Pupil dynamics during bistable motion perception. *Journal of Vision, 9*(7). doi: 10.1167/9.7.10

Huppert, J. D., & Sanderson, W. C. (2010). Psychotherapy for generalized anxiety disorder. In D. J. Stein, E. Hollander & B. O. Rothbaum (Eds.), *Textbook of anxiety disorders (2nd ed.).* (pp. 219-238). Arlington, VA US: American Psychiatric Publishing, Inc.

Jackson, P. L., Rainville, P., & Decety, J. (2006). To what extent do we share the pain of others? Insight from the neural bases of pain empathy. *Pain, 125*(1-2), 5-9. doi: 10.1016/j.pain.2006.09.013

Karama, S., Lecours, A. R., Leroux, J. M., Bourgouin, P., Beaudoin, G., Joubert, S., & Beauregard, M. (2002). Areas of brain activation in males and females during viewing of erotic film excerpts. *Human brain mapping, 16*(1), 1-13.

Kent, J. M., & Rauch, S. L. (2003). Neurocircuitry of anxiety disorders. *Curr Psychiatry Rep, 5*(4), 266-273.

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of general psychiatry, 62*(6), 617-627.

Kim, M. J., Chey, J., Chung, A., Bae, S., Khang, H., Ham, B., . . . Lyoo, I. K. (2008). Diminished rostral anterior cingulate activity in response to threat-related events in posttraumatic stress disorder. *J Psychiatr Res, 42*(4), 268-277. doi: 10.1016/j.jpsychires.2007.02.003

Knutson, B., Adams, C. M., Fong, G. W., & Hommer, D. (2001). Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 21*(16), RC159.

Knutson, B., & Greer, S. M. (2008). Anticipatory affect: neural correlates and consequences for choice. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 363*(1511), 3771-3786. doi: 10.1098/rstb.2008.0155

Knutson, B., & Peterson, R. (2005). Neurally reconstructing expected utility. *Games and Economic Behavior, 52*(2), 305-315. doi: 10.1016/j.geb.2005.01.002

Knutson, B., Taylor, J., Kaufman, M., Peterson, R., & Glover, G. (2005). Distributed neural representation of expected value. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 25*(19), 4806-4812. doi: 10.1523/JNEUROSCI.0642-05.2005

Knutson, B., & Wimmer, G. E. (2007). Reward: Neural Circuitry for Social Valuation. In E. Harmon-Jones & P. Winkielman (Eds.), *Social neuroscience: Integrating biological and psychological explanations of social behavior.* (pp. 157-175). New York, NY US: Guilford Press.

Krain, A. L., Gotimer, K., Hefton, S., Ernst, M., Castellanos, F. X., Pine, D. S., & Milham, M. P. (2008). A functional magnetic resonance imaging investigation of uncertainty in adolescents with anxiety disorders. *Biol Psychiatry, 63*(6), 563-568. doi: 10.1016/j.biopsych.2007.06.011

LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E., & Phelps, E. A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron, 20*(5), 937-945.

Ladouceur, R., Dugas, M. J., Freeston, M. H., Rhéaume, J., Blais, F., Boisvert, J., . . . Thibodeau, N. (1999). Specificity of Generalized Anxiety Disorder Symptoms and Processes. *Behavior Therapy, 30*(2), 191-207. doi: 10.1016/s0005-7894(99)80003-3

Ladouceur, R., Gosselin, P., & Dugas, M. J. (2000). Experimental manipulation of intolerance of uncertainty: a study of a theoretical model of worry. *Behav Res Ther, 38*(9), 933-941.

Lane, R. D. (1999). *Functional neuroanatomy of pleasant and unpleasant emotion.* (Ph.D. 9927500), The University of Arizona, United States -- Arizona. Retrieved from https://libproxy.cc.stonybrook.edu/login?url=http://search.proquest.com/docview/304520113?accountid=14172 ProQuest Dissertations & Theses (PQDT) database.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1998). Emotion, motivation, and anxiety: Brain mechanisms and psychophysiology. *Biological Psychiatry, 44*(12), 1248-1263. doi: 10.1016/s0006-3223(98)00275-3

Lang, P. J., Bradley, M. M., Fitzsimmons, J. R., Cuthbert, B. N., Scott, J. D., Moulder, B., & Nangia, V. (1998). Emotional arousal and activation of the visual cortex: an fMRI analysis. *Psychophysiology, 35*(2), 199-210.

Lanius, R. A., Williamson, P. C., Densmore, M., Boksman, K., Gupta, M. A., Neufeld, R. W., . . . Menon, R. S. (2001). Neural correlates of traumatic memories in posttraumatic stress disorder: a functional MRI investigation. *Am J Psychiatry, 158*(11), 1920-1922.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review Of Neuroscience, 23*, 155-184. doi: 10.1146/annurev.neuro.23.1.155

Liberzon, I., Phan, K. L., Decker, L. R., & Taylor, S. F. (2003). Extended amygdala and emotional salience: a PET activation study of positive and negative affect. *Neuropsychopharmacology, 28*(4), 726-733. doi: 10.1038/sj.npp.1300113

Linnman, C., Rougemont-Bucking, A., Beucke, J. C., Zeffiro, T. A., & Milad, M. R. (2011). Unconditioned responses and functional fear networks in human classical conditioning. *Behavioural brain research, 221*(1), 237-245. doi: 10.1016/j.bbr.2011.02.045

Lissek, S. (2012). Toward an account of clinical anxiety predicated on basic, neurally mapped mechanisms of Pavlovian fear-learning: the case for conditioned overgeneralization. *Depress Anxiety, 29*(4), 257-263. doi: 10.1002/da.21922

Lissek, S., Biggs, A. L., Rabin, S. J., Cornwell, B. R., Alvarez, R. P., Pine, D. S., & Grillon, C. (2008). Generalization of conditioned fear-potentiated startle in humans: experimental validation and clinical relevance. *Behav Res Ther, 46*(5), 678-687. doi: 10.1016/j.brat.2008.02.005

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behav Res Ther, 43*(11), 1391-1424. doi: 10.1016/j.brat.2004.10.007

Lissek, S., Rabin, S., Heller, R. E., Lukenbaugh, D., Geraci, M., Pine, D. S., & Grillon, C. (2010). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *The American Journal Of Psychiatry, 167*(1), 47-55.

Lorberbaum, J. P., Kose, S., Johnson, M. R., Arana, G. W., Sullivan, L. K., Hamner, M. B., . . . George, M. S. (2004). Neural correlates of speech anticipatory anxiety in generalized social phobia. *Neuroreport, 15*(18), 2701-2705.

Lovero, K. L., Simmons, A. N., Aron, J. L., & Paulus, M. P. (2009). Anterior insular cortex anticipates impending stimulus significance. *NeuroImage, 45*(3), 976-983. doi: 10.1016/j.neuroimage.2008.12.070

Maslowsky, J., Mogg, K., Bradley, B. P., McClure, E. B., Ernst, M., Pine, D. S., & Monk, C. S. (2010). A preliminary investigation of neural correlates of treatment in adolescents with generalized anxiety disorder. *Journal Of Child And Adolescent Psychopharmacology, 20*(2), 105-111. doi: 10.1089/cap.2009.0049

Mataix-Cols, D., An, S. K., Lawrence, N. S., Caseras, X., Speckens, A., Giampietro, V., . . . Phillips, M. L. (2008). Individual differences in disgust sensitivity modulate neural responses to aversive/disgusting stimuli. *The European Journal Of Neuroscience, 27*(11), 3050-3058. doi: 10.1111/j.1460-9568.2008.06311.x

Mathews, A. (1990). Why worry? The cognitive function of anxiety. *Behav Res Ther, 28*(6), 455-468. doi: 10.1016/0005-7967(90)90132-3

Mathews, A., Richards, A., & Eysenck, M. (1989). Interpretation of homophones related to threat in anxiety states. *J Abnorm Psychol, 98*(1), 31-34.

Mauss, I. B., Bunge, S. A., & Gross, J. J. (2007). Automatic emotion regulation. *Social and Personality Psychology Compass, 1*(1), 146-167. doi: 10.1111/j.1751-9004.2007.00005.x

McClure, E. B., Adler, A., Monk, C. S., Cameron, J., Smith, S., Nelson, E. E., . . . Pine, D. S. (2007). fMRI predictors of treatment outcome in pediatric anxiety disorders. *Psychopharmacology (Berl), 191*(1), 97-105. doi: 10.1007/s00213-006-0542-9

McClure, E. B., Monk, C. S., Nelson, E. E., Parrish, J. M., Adler, A., Blair, R. J., . . . Pine, D. S. (2007). Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. *Archives of general psychiatry, 64*(1), 97-106. doi: 10.1001/archpsyc.64.1.97

McClure, S. M., York, M. K., & Montague, P. R. (2004). The neural substrates of reward processing in humans: the modern role of FMRI. *Neuroscientist, 10*(3), 260-268. doi: 10.1177/1073858404263526

Mechias, M. L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *NeuroImage, 49*(2), 1760-1768. doi: 10.1016/j.neuroimage.2009.09.040

Medford, N., & Critchley, H. D. (2010). Conjoint activity of anterior insular and anterior cingulate cortex: awareness and response. *Brain Structure & Function, 214*(5-6), 535-549. doi: 10.1007/s00429-010-0265-x

Mennin, D. S., Heimberg, R. G., & Turk, C. L. (2004). Clinical Presentation and Diagnostic Features. In R. G. Heimberg, C. L. Turk & D. S. Mennin (Eds.), *Generalized anxiety disorder: Advances in research and practice.* (pp. 3-28). New York, NY US: Guilford Press.

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: A network model of insula function. *Brain Structure & Function, 214*(5-6), 655-667. doi: 10.1007/s00429-010-0262-0

Meyer, T. J., Miller, M. L., Metzger, R. L., & Borkovec, T. D. (1990). Development and validation of the Penn State Worry Questionnaire. *Behav Res Ther, 28*(6), 487-495. doi: 10.1016/0005-7967(90)90135-6

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry, 66*(12), 1075-1082. doi: 10.1016/j.biopsych.2009.06.026

Milad, M. R., Rauch, S. L., Pitman, R. K., & Quirk, G. J. (2006). Fear extinction in rats: Implications for human brain imaging and anxiety disorders. *Biological Psychology, 73*(1), 61-71. doi: 10.1016/j.biopsycho.2006.01.008

Milad, M. R., Wright, C. I., Orr, S. P., Pitman, R. K., Quirk, G. J., & Rauch, S. L. (2007). Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol Psychiatry, 62*(5), 446-454. doi: 10.1016/j.biopsych.2006.10.011

Mineka, S., & Oehlberg, K. (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*(3), 567-580. doi: 10.1016/j.actpsy.2007.11.007

Mogg, K., & Bradley, B. P. (2005). Attentional Bias in Generalized Anxiety Disorder Versus Depressive Disorder. *Cognitive Therapy and Research, 29*(1), 29-45. doi: 10.1007/s10608-005-1646-y

Molina, S., & Borkovec, T. D. (1994). The Penn State Worry Questionnaire: Psychometric properties and associated characteristics. In G. C. L. Davey & F. Tallis (Eds.), *Worrying: Perspectives on theory, assessment and treatment.* (pp. 265-283). Oxford England: John Wiley & Sons.

Monk, C. S., Nelson, E. E., McClure, E. B., Mogg, K., Bradley, B. P., Leibenluft, E., . . . Pine, D. S. (2006). Ventrolateral prefrontal cortex activation and attentional bias in response to angry faces in adolescents with generalized anxiety disorder. *Am J Psychiatry, 163*(6), 1091-1097. doi: 10.1176/appi.ajp.163.6.1091

Monk, C. S., Telzer, E. H., Mogg, K., Bradley, B. P., Mai, X., Louro, H. M., . . . Pine, D. S. (2008). Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. *Archives of general psychiatry, 65*(5), 568-576. doi: 10.1001/archpsyc.65.5.568

Morgan, M. A., & LeDoux, J. E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behavioral neuroscience, 109*(4), 681-688.

Morris, J. S., & Dolan, R. J. (2004). Dissociable amygdala and orbitofrontal responses during reversal fear conditioning. *NeuroImage, 22*(1), 372-380. doi: 10.1016/j.neuroimage.2004.01.012

Murphy, F. C., Nimmo-Smith, I., & Lawrence, A. D. (2003). Functional neuroanatomy of emotions: A meta-analysis. *Cogn Affect Behav Neurosci, 3*(3), 207-233. doi: 10.3758/cabn.3.3.207

Naragon-Gainey, K. (2010). Meta-analysis of the relations of anxiety sensitivity to the depressive and anxiety disorders. *Psychological Bulletin, 136*(1), 128-150. doi: 10.1037/a001805510.1037/a0018055.supp (Supplemental)

Nichols, T., Brett, M., Andersson, J., Wager, T., & Poline, J. B. (2005). Valid conjunction inference with the minimum statistic. *NeuroImage, 25*(3), 653-660. doi: 10.1016/j.neuroimage.2004.12.005

Nitschke, J. B., Sarinopoulos, I., Mackiewicz, K. L., Schaefer, H. S., & Davidson, R. J. (2006). Functional neuroanatomy of aversion and its anticipation. *NeuroImage, 29*(1), 106-116. doi: 10.1016/j.neuroimage.2005.06.068

Nitschke, J. B., Sarinopoulos, I., Oathes, D. J., Johnstone, T., Whalen, P. J., Davidson, R. J., & Kalin, N. H. (2009). Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. *Am J Psychiatry, 166*(3), 302-310. doi: 10.1176/appi.ajp.2008.07101682

O'Doherty, J. P. (2004). Reward representations and reward-related learning in the human brain: insights from neuroimaging. *Current Opinion in Neurobiology, 14*(6), 769-776. doi: 10.1016/j.conb.2004.10.016

O'Doherty, J. P., Deichmann, R., Critchley, H. D., & Dolan, R. J. (2002). Neural responses during anticipation of a primary taste reward. *Neuron, 33*(5), 815-826.

Ochsner, K. N., Bunge, S. A., Gross, J. J., & Gabrieli, J. D. (2002). Rethinking feelings: an FMRI study of the cognitive regulation of emotion. *Journal of cognitive neuroscience, 14*(8), 1215-1229. doi: 10.1162/089892902760807212

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends Cogn Sci, 9*(5), 242-249. doi: 10.1016/j.tics.2005.03.010

Ochsner, K. N., Knierim, K., Ludlow, D. H., Hanelin, J., Ramachandran, T., Glover, G., & Mackey, S. C. (2004). Reflecting upon feelings: an fMRI study of neural systems supporting the attribution of emotion to self and other. *Journal of cognitive neuroscience, 16*(10), 1746-1772.

Olatunji, B. O., Ciesielski, B. G., Armstrong, T., Zhao, M., & Zald, D. H. (2011). Making something out of nothing: neutral content modulates attention in generalized anxiety disorder. *Depress Anxiety, 28*(5), 427-434. doi: 10.1002/da.20806

Paulus, M. P., & Stein, M. B. (2006). An Insular View of Anxiety. *Biological Psychiatry, 60*(4), 383-387. doi: 10.1016/j.biopsych.2006.03.042

Peterson, R. A., & Reiss, S. (1992). *Anxiety Sensitivity Index Manual (2nd ed.)*. Worthington, OH: International Diagnostic Systems.

Phelps, E. A., Delgado, M. R., Nearing, K. I., & LeDoux, J. E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron, 43*(6), 897-905. doi: 10.1016/j.neuron.2004.08.042

Phelps, E. A., O'Connor, K. J., Gatenby, J. C., Gore, J. C., Grillon, C., & Davis, M. (2001). Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience, 4*(4), 437-441. doi: 10.1038/86110

Phillips, M. L., Young, A. W., Senior, C., Brammer, M., Andrew, C., Calder, A. J., . . . David, A. S. (1997). A specific neural substrate for perceiving facial expressions of disgust. *Nature, 389*(6650), 495-498. doi: 10.1038/39051

Quirk, G. J., Russo, G. K., Barron, J. L., & Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 20*(16), 6225-6231.

Rauch, S. L., Shin, L. M., & Wright, C. I. (2003). Neuroimaging studies of amygdala function in anxiety disorders. *Ann N Y Acad Sci, 985*, 389-410.

Reinecke, A., Becker, E. S., Hoyer, J., & Rinck, M. (2010). Generalized implicit fear associations in generalized anxiety disorder. *Depress Anxiety, 27*(3), 252-259. doi: 10.1002/da.20662

Ressler, K. J., Rothbaum, B. O., Tannenbaum, L., Anderson, P., Graap, K., Zimand, E., . . . Davis, M. (2004). Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Archives of general psychiatry, 61*(11), 1136-1144. doi: 10.1001/archpsyc.61.11.1136

Rolls, E. T. (2000). Precis of The brain and emotion. *Behav Brain Sci, 23*(2), 177-191; discussion 192-233.

Sander, D., Grafman, J., & Zalla, T. (2003). The human amygdala: an evolved system for relevance detection. *Reviews In The Neurosciences, 14*(4), 303-316.

Schiller, D., Levy, I., Niv, Y., LeDoux, J. E., & Phelps, E. A. (2008). From fear to safety and back: reversal of fear in the human brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 28*(45), 11517-11525. doi: 10.1523/JNEUROSCI.2265-08.2008

Sehlmeyer, C., Schöning, S., Zwitserlood, P., Pfleiderer, B., Kircher, T., Arolt, V., & Konrad, C. (2009). Human fear conditioning and extinction in neuroimaging: a systematic review. *Plos One, 4*(6), e5865-e5865. doi: 10.1371/journal.pone.0005865

Shaban, H., Humeau, Y., Herry, C., Cassasus, G., Shigemoto, R., Ciocchi, S., . . . Luthi, A. (2006). Generalization of amygdala LTP and conditioned fear in the absence of presynaptic inhibition. *Nature Neuroscience, 9*(8), 1028-1035. doi: 10.1038/nn1732

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., . . . Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal Of Clinical Psychiatry, 59 Suppl 20*, 22-33.

Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology, 35*(1), 169-191. doi: 10.1038/npp.2009.83

Shin, L. M., Orr, S. P., Carson, M. A., Rauch, S. L., Macklin, M. L., Lasko, N. B., . . . Pitman, R. K. (2004). Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. *Archives of general psychiatry, 61*(2), 168-176. doi: 10.1001/archpsyc.61.2.168

Shin, L. M., Wright, C. I., Cannistraro, P. A., Wedig, M. M., McMullin, K., Martis, B., . . . Rauch, S. L. (2005). A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic

stress disorder. *Archives of general psychiatry, 62*(3), 273-281. doi: 10.1001/archpsyc.62.3.273

Simmons, A. N., Matthews, S. C., Stein, M. B., & Paulus, M. P. (2004). Anticipation of emotionally aversive visual stimuli activates right insula. *Neuroreport, 15*(14), 2261-2265.

Simmons, A. N., Stein, M. B., Strigo, I. A., Arce, E., Hitchcock, C., & Paulus, M. P. (2011). Anxiety positive subjects show altered processing in the anterior insula during anticipation of negative stimuli. *Human brain mapping, 32*(11), 1836-1846. doi: 10.1002/hbm.21154

Simmons, A. N., Strigo, I. A., Matthews, S. C., Paulus, M. P., & Stein, M. B. (2006). Anticipation of Aversive Visual Stimuli Is Associated With Increased Insula Activation in Anxiety-Prone Subjects. *Biological Psychiatry, 60*(4), 402-409. doi: 10.1016/j.biopsych.2006.04.038

Spielberger, C. D., Gorsuch, R. L., Lushene, R. E., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the state-trait anxiety inventory (Form Y)*. Palo Alto, CA: Consulting Psychologists Press.

Straube, T., Mentzel, H. J., & Miltner, W. H. (2005). Common and distinct brain activation to threat and safety signals in social phobia. *Neuropsychobiology, 52*(3), 163-168. doi: 10.1159/000087987

Straube, T., Mentzel, H. J., & Miltner, W. H. (2007). Waiting for spiders: brain activation during anticipatory anxiety in spider phobics. *NeuroImage, 37*(4), 1427-1436. doi: 10.1016/j.neuroimage.2007.06.023

Thakral, P. P., & Slotnick, S. D. (2009). The role of parietal cortex during sustained visual spatial attention. *Brain Research, 1302*, 157-166. doi: 10.1016/j.brainres.2009.09.031

Tillfors, M., Furmark, T., Marteinsdottir, I., & Fredrikson, M. (2002). Cerebral blood flow during anticipation of public speaking in social phobia: a PET study. *Biol Psychiatry, 52*(11), 1113-1119.

Tromp, D. P. M., Grupe, D. W., Oathes, D. J., McFarlin, D. R., Hernandez, P. J., Kral, T. R. A., . . . Nitschke, J. B. (2012). Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. *Archives of general psychiatry, 69*(9), 925-934.

Urry, H. L., van Reekum, C. M., Johnstone, T., Kalin, N. H., Thurow, M. E., Schaefer, H. S., . . . Davidson, R. J. (2006). Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 26*(16), 4415-4425. doi: 10.1523/JNEUROSCI.3215-05.2006

van Overveld, W. J. M., de Jong, P. J., Peters, M. L., Cavanagh, K., & Davey, G. C. L. (2006). Disgust propensity and disgust sensitivity: Separate constructs that are differentially related to specific fears. *Personality and Individual Differences, 41*(7), 1241-1252. doi: 10.1016/j.paid.2006.04.021

Vervliet, B., Kindt, M., Vansteenwegen, D., & Hermans, D. (2010). Fear generalization in humans: impact of verbal instructions. *Behav Res Ther, 48*(1), 38-43. doi: 10.1016/j.brat.2009.09.005

Vink, M., Kahn, R. S., Raemaekers, M., van den Heuvel, M., Boersma, M., & Ramsey, N. F. (2005). Function of striatum beyond inhibition and execution of motor responses. *Human brain mapping, 25*(3), 336-344. doi: 10.1002/hbm.20111

Vuilleumier, P., & Driver, J. (2007). Modulation of visual processing by attention and emotion: windows on causal interactions between human brain regions. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 362*(1481), 837-855. doi: 10.1098/rstb.2007.2092

Vytal, K., & Hamann, S. (2010). Neuroimaging support for discrete neural correlates of basic emotions: a voxel-based meta-analysis. *Journal of cognitive neuroscience, 22*(12), 2864-2885. doi: 10.1162/jocn.2009.21366

Walker, D. L., Ressler, K. J., Lu, K. T., & Davis, M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 22*(6), 2343-2351.

Walter, B., Blecker, C., Kirsch, P., Sammer, G., Schienle, A., & Stark, R. (2003). *MARINA: an easy to use tool for the creation of MAsks for Region of INterest Analyses*. In: Paper presented at the Ninth International Conference on Functional Mapping of the Human Brain. NY, New York

Watson, D., & Clark, L. A. (1991). *The Mood and Anxiety Symptom Questionnaire*: Unpublished manuscript, University of Iowa City.

Watson, D., Weber, K., Assenheimer, J. S., Clark, L. A., Strauss, M. E., & McCormick, R. A. (1995). Testing a tripartite model: I. Evaluating the convergent and discriminant validity of anxiety and depression symptom scales. *J Abnorm Psychol, 104*(1), 3-14. doi: 10.1037/0021-843x.104.1.3

Whalen, P. J., Johnstone, T., Somerville, L. H., Nitschke, J. B., Polis, S., Alexander, A. L., . . . Kalin, N. H. (2008). A functional magnetic resonance imaging predictor of treatment

response to venlafaxine in generalized anxiety disorder. *Biological Psychiatry, 63*(9), 858-863. doi: 10.1016/j.biopsych.2007.08.019

Wik, G., Fredrikson, M., & Fischer, H. (1996). Cerebral correlates of anticipated fear: A PET study of specific phobia. *International Journal of Neuroscience, 87*(3-4), 267-276. doi: 10.3109/00207459609070845

Wild, J. M., & Blampied, N. M. (1972). Hippocampal lesions and stimulus generalization in rats. *Physiol Behav, 9*(4), 505-511.

Wittchen, H. U., Zhao, S., Kessler, R. C., & Eaton, W. W. (1994). DSM-III-R generalized anxiety disorder in the National Comorbidity Survey. *Archives of general psychiatry, 51*(5), 355-364.

Wright, P., He, G., Shapira, N. A., Goodman, W. K., & Liu, Y. (2004). Disgust and the insula: fMRI responses to pictures of mutilation and contamination. *Neuroreport, 15*(15), 2347-2351.

Zinbarg, R. E., & Barlow, D. H. (1996). Structure of anxiety and the anxiety disorders: a hierarchical model. *J Abnorm Psychol, 105*(2), 181-193.