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Neural Substrates of Fear Extinction: A Potential Predictor of Exposure Success

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by

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DEDICATION

To my parents, who first nurtured my curiosity.

To my husband, who keeps me grounded.

To my son, who puts it all in perspective.

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ABSTRACT OF THE DISSERTATION

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by

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Despite the efficacy of exposure therapy for anxiety, there are individual differences in outcomes that are not well understood. Fear extinction learning, in which a previously feared stimulus is no longer paired with negative outcomes, is the hypothesized mechanism of exposure. Relative to healthy adults, anxious individuals show greater resistance to fear extinction learning and altered amygdala and ventromedial prefrontal cortex (vmPFC) activation. The goals of this dissertation were

therefore to test associations between neural bases of fear extinction and (1) anxiety severity, as well as (2) exposure efficacy.

Twenty-four adults with public speaking anxiety completed a fear conditioning task during functional magnetic resonance imaging (fMRI). During fear acquisition, one neutral stimulus (CS+) was paired with a loud scream and another (CS-) was presented but never paired. During fear extinction, both CS+ and CS- were presented without any aversive stimulus. In a subsequent exposure session, participants completed four five-minute speeches. Finally, participants completed an anxiety assessment by phone two weeks later. Robust regression analyses were used to relate neural correlates of fear extinction learning to baseline anxiety and anxiety reduction following exposure.

Ratings of negative valence and arousal to the CS+ increased following fear acquisition and diminished following extinction. Individuals who rated the CS+ more negatively showed greater amygdala activation during acquisition and extinction, and also reported less anxiety reduction following exposure. Individuals with greater baseline anxiety severity had greater activation in dorsomedial PFC. Finally, individuals with greater vmPFC and less amygdala activation during extinction reported greater anxiety reduction from baseline to follow-up.

Those individuals who, by self-report and neural activation, demonstrated better extinction learning also reported greater anxiety reduction following an exposure intervention. This is the first time that the theoretical link between extinction learning and exposure efficacy has been demonstrated. The results suggest that

individuals whose brain activation dynamically adjusts to the presence or absence of aversive consequences may benefit most from brief exposure therapy, providing an important step toward the mechanistic understanding of exposure. Future work should examine whether fear extinction can reliably predict clinical outcomes and be used as a prognostic test to guide treatment decisions.

THESIS

Introduction

Anxiety disorders are highly prevalent (Kessler, Chiu, Demler, Merikangas, & Walters, 2005a) and significantly impairing (Mendlowicz & Stein, 2000). Although efficacious treatments for anxiety exist, they are not universally successful. In fact, the prevalence of anxiety disorders has increased over the last several decades, despite simultaneous increases in the availability of effective treatments (Kessler et al., 2005b). At the same time, functional neuroimaging has led to significant gains in understanding the biological bases of anxiety disorders. However, this increase in knowledge has not directly translated to improved clinical outcomes, and these conditions continue to demonstrate high prevalence, functional impairment, and reduced quality of life. One important method by which neuroimaging could improve clinical care is through the generation of predictive models that use neuroimaging data to guide clinical decision-making. However, existing research that moves the field in this direction has been extremely sparse. This line of research would be aided by a mechanistically-grounded understanding of individual differences in treatment response.

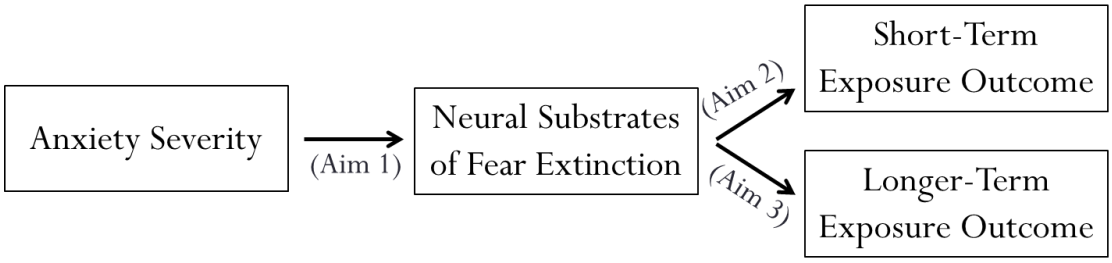

Figure 1. Conceptual model illustrating the aims of the study.

The present dissertation study aims to understand these differences in treatment response in anxiety patients. Specifically, a major mechanism of cognitive behavioral therapy (CBT), an empirically-supported treatment for anxiety, is fear extinction learning in the form of therapeutic exposure. However, despite the theoretical links between fear extinction learning and exposure, individual differences in extinction learning have not been examined as predictors of exposure response. The present study sought to examine the neural substrates of fear extinction in a group of individuals with public speaking anxiety. Specifically, the relationships between these neural substrates and (1) public speaking anxiety severity, (2) short-term and (3) longer-term reduction of anxiety following exposure were examined (Figure 1).

Public Speaking Anxiety

Public speaking anxiety, a subtype of social phobia (Stein, Torgrud, & Walker, 2000), is a useful model system for understanding anxiety treatment. Phobias have long been used as an analogue system to understand anxiety disorders as a class (Bernstein & Paul, 1971), because although individuals with specific phobias are typically higher functioning and have less comorbidity than those with other anxiety disorders (Kessler, Stein, & Berglund, 1998), the same mechanism is thought to underlie the development and treatment of phobias and other anxiety disorders (Mineka & Oehlberg, 2008). Furthermore, lifetime prevalence rates of social phobia have been estimated to be greater than 13% (Magee, Eaton, Wittchen, McGonagle, & Kessler, 1996), and as many as 30% of the population report a fear of public speaking (Kessler et al., 1998). The high prevalence and relatively straightforward clinical

presentation makes public speaking anxiety an excellent analogue for understanding predictors of anxiety treatment outcome.

Exposure Therapy

Among available treatments for anxiety, CBT has consistently shown large effect sizes, with treatment gains maintained at follow-up (Deacon & Abramowitz, 2004). The question of which components of CBT are necessary and/or sufficient has been the subject of much debate (e.g., Longmore & Worrell, 2007). Although there is some evidence that cognitive change may mediate therapy outcomes (Hofmann, 2004), theoretical formulations and empirical evidence from dismantling studies have indicated that exposure alone is often equally effective to exposure combined with cognitive therapy, consistent with the notion that exposure is the central mechanism of CBT for anxiety (Feske & Chambless, 1995; Foa, Rothbaum, & Furr, 2003; Longmore & Worrell, 2007).

Therapeutic exposure involves repeated deliberate engagement with a feared stimulus, such as a formal speech, allowing anxiety reactions to reduce over time. A better understanding of the correlates of exposure success is an important translational research goal. The current study used a massed exposure protocol (i.e., multiple exposures in a single session) to identify potential predictors of short- and longer-term exposure success. Although exposures are typically spaced throughout multiple weeks of therapy, the massed exposure protocol is a useful laboratory analogue of the exposure therapy process. Massed exposure has been shown to yield robust short-term anxiety reduction, with variability in return of fear at follow-up (Tsao & Craske,

2000), and prior studies have examined correlates of exposure success in public speaking anxiety using massed exposure (Vasey, Harbaugh, Buffington, Jones, & Fazio, 2012).

Fear Conditioning

Therapeutic exposure is based on principles of fear extinction learning (Craske et al., 2008). In other words, exposure to a feared stimulus without the feared outcome occurring allows the patient to learn new expectancies, thereby reducing anxiety. Fear extinction also provides a key translational link to animal models of pathological anxiety. In both human and animal models, fear extinction is typically studied with a conditioning paradigm involving two phases: fear acquisition and fear extinction. During fear acquisition, a previously neutral stimulus such as a visually displayed image (the conditioned stimulus, or CS), is paired with an aversive stimulus such as a loud noise or mild shock (the unconditioned stimulus, or US). This results in a conditioned fear response to the CS. During the extinction phase this fearful reaction is diminished by repeated presentations of the CS without the US, analogous to the presumed mechanism of exposure therapy.

This classic version of fear conditioning can be modified to examine discriminant learning. In discriminant fear conditioning, two neutral stimuli are presented. One stimulus (the CS+) is paired with the US as described above, while the other (the CS-) is presented during the task but never paired with the US. Differences between responses to the CS+ and the CS- are compared, with the CS+ expected to

yield a conditioned fear response that is then extinguished, while responses to the CS- are expected to show little change throughout the task.

Although early fear extinction theories posited that extinction necessitated un-learning previously established CS-US associations, more recent accounts suggest that fear extinction involves new learning that competes with the previously learned association, leaving the conditioned fear memory intact (Bouton, 2002; Craske et al., 2008; Myers & Davis, 2002). Indeed, a return of fear responding is sometimes seen after the passage of time (spontaneous recovery), in new contexts (renewal), or if the individual is re-exposed to the US alone (reinstatement) (Hermans, Craske, Mineka, & Lovibond, 2006; Mineka, Mystkowski, Hladek, & Rodriguez, 1999). Thus, successful and robust fear extinction learning is thought to require not only the acquisition of a new CS-safety association but also inhibition of the previously learned and likely salient CS-threat association (Craske et al., 2008; Davis, Falls, & Gewirtz, 2000).

Fear conditioning in anxious adults. Many theoretical models of anxiety are based on fear conditioning principles, positing that highly anxious individuals more readily learn threat associations and have difficulty extinguishing these associations in laboratory fear conditioning tasks (Mineka & Oehlberg, 2008). Early formulations of this theoretical approach held that problematic anxiety reactions were learned responses directly caused by prior fear conditioning-like experiences (reviewed in Rachman, 1991). Although adverse experiences that may have initiated conditioned fear responding are sometimes reported by patients, such events are neither necessary (with the exception of post-traumatic stress disorder) nor sufficient for the acquisition

of anxiety pathology (Graham & Milad, 2011). Nevertheless, a meta-analysis found support for more robust acquisition of fear in anxious individuals, suggesting that this feature may play a role in the etiology of anxiety disorders (Lissek et al., 2005). In particular, anxious individuals may fail to inhibit fear responses in the presence of safety cues (Davis et al., 2000), leading to enhanced fear responding to the CS- in discriminant fear conditioning (Lissek et al., 2005).

Anxiety disorders are also thought to be maintained by a failure of fear extinction (Mineka & Oehlberg, 2008; Rachman, 1991). This perspective is supported by the development and subsequent success of exposure-based therapies for anxiety disorders, which seek to remedy deficiencies in fear extinction by encouraging and reinforcing extinction via safe exposure to a feared stimulus. Consistent with this theory, a meta-analysis found that anxious individuals do show greater resistance to fear extinction relative to healthy adults (Lissek et al., 2005). A large-scale prospective study has recently implicated fear extinction learning deficits in the acquisition of anxiety symptoms following trauma exposure (Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013). Deficits in fear extinction could therefore underlie both vulnerability for as well as ongoing maintenance of anxiety disorders (Rauch, Shin, & Phelps, 2006).

Prior research on fear conditioning specifically in social phobia has been mixed, with some studies finding no differences in fear conditioning (Schneider et al., 1999) and others finding stronger fear acquisition (Lissek et al., 2008; Pejic, Hermann, Vaitl, & Stark, 2013), as well as greater resistance to extinction and poorer

discrimination between a CS+ and CS- (Hermann, Ziegler, Birbaumer, & Flor, 2002). Taken together, there is theoretical and empirical evidence to suggest abnormal fear conditioning in social phobia. However, although therapeutic exposure is based on fear extinction principles, associations between fear extinction learning and exposure efficacy have not been investigated. One would expect that anxious individuals with greater resistance to extinction learning in a laboratory task would also be more resistant to therapeutic exposure techniques, but this has not been directly tested.

Neural Correlates of Fear Extinction

Functional neuroimaging, specifically functional magnetic resonance imaging (fMRI), non-invasively indexes regional neuronal activation underlying key psychological processes such as fear extinction learning. Functional neuroimaging may be particularly useful because it is thought to quantify the underlying biological disease state of psychological conditions. Given that data on differential fear conditioning in social phobia have been somewhat mixed when examining behavioral and physiological indicators, utilizing fMRI to index fear extinction learning may provide a more direct and reliable indicator of the underlying mechanism. fMRI can therefore be used both to identify potential neural predictors of outcome, as well as to discover underlying mechanisms through which psychological constructs, such as greater anxiety severity, relate to treatment outcome.

Neural bases of fear conditioning in animal models. Decades of work have supported the role of the amygdala in the representation of fear and the integration of information during fear acquisition (e.g., Blair, Schafe, Bauer, Rodrigues, & LeDoux,

2001; Kim & Jung, 2006; LeDoux, 1998). Lesion studies in rats have indicated that the amygdala, particularly its lateral and central nuclei, is necessary for fear acquisition (Maren, 2001; R. Phillips & LeDoux, 1992). Other studies have suggested that the basal nucleus of the amygdala is necessary for the expression of previously conditioned fear (Anglada-Figueroa & Quirk, 2005), and that inhibitory circuits within the amygdala are necessary for the expression of fear extinction (Likhtik, Popa, Apergis-Schoute, Fidacaro, & Paré, 2008). The amygdala therefore plays a prominent role in fear acquisition, expression, and extinction.

Animal work has also implicated the medial prefrontal cortex (PFC), in fear conditioning. Rodent medial PFC is often divided into prelimbic and infralimbic cortices; in humans dorsal ACC is roughly homologous to prelimbic cortex and ventromedial PFC (vmPFC), which consists primarily of medial OFC and ventral ACC, is roughly homologous to infralimbic cortex. Animal studies have implicated the prelimbic cortex in fear expression and acquisition (Burgos-Robles, Vidal-Gonzalez, & Quirk, 2009). Furthermore, sustained prelimbic activation following extinction training has been associated with poorer longer term extinction learning (Burgos-Robles et al., 2009). In contrast, the infralimbic cortex has been strongly implicated in successful fear extinction learning. Lesion studies in rats have shown that damage to the infralimbic cortex impairs extinction learning (Morgan, Romanski, & LeDoux, 1993). Complementary work using single-unit recording in rats found that greater firing in infralimbic neurons was associated with less return of fear following extinction (Milad & Quirk, 2002). Taken together, the evidence suggests that the

rodent homologue of the vmPFC is involved in storing new associations and inhibiting previously learned fear relationships (R. McNally, 2007).

Neural bases of fear conditioning in healthy adults. The advent of non-invasive functional neuroimaging techniques such as fMRI have allowed the neural bases of fear conditioning to be studied in adult humans, with results generally paralleling findings in animals. Consistent with animal studies, amygdala activation has been reliably observed in humans during both fear acquisition and extinction, particularly early in each phase of conditioning (Büchel, Morris, Dolan, & Friston, 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). Amygdala activation during both fear acquisition and extinction has also been associated with autonomic conditioned fear responding, lending further support to its role in fear expression (Phelps, Delgado, Nearing, & LeDoux, 2004). The dorsal ACC has also been shown to be active during fear acquisition and often early extinction, and is thought to signal positively to the amygdala (Linnman et al., 2012b).

Although the amygdala and dorsal ACC play a prominent role in fear conditioning, other brain regions have been implicated as well. The anterior insular cortex has been implicated in integrating interoceptive information to predict expected outcomes (Paulus & Stein, 2006), a process with clear relevance for fear conditioning. Accordingly, activation in the anterior insula has often been observed during fear acquisition and extinction in human neuroimaging studies (Büchel et al., 1998; Phelps et al., 2001; Sehlmeyer et al., 2009). In addition, periaqueductal grey (PAG) is a midbrain structure that has been implicated in the experience of pain and fear, and

receives projections from both the amygdala and vmPFC (Linnman, Moulton, Barmettler, Becerra, & Borsook, 2012a). Direct stimulation of PAG induces a fear response, and such stimulation has been used as an unconditioned stimulus in animal studies of fear conditioning (Di Scala, Mana, Jacobs, & Phillips, 1987). The PAG is therefore likely to track acquired fear to the conditioned stimulus, and may also play a role in extinction (G. McNally, Pigg, & Weidemann, 2004).

Studies of fear extinction in humans have also strongly paralleled the animal literature and have highlighted the role of the vmPFC. Ventromedial PFC activation has been demonstrated during fear extinction in humans (Gottfried & Dolan, 2004; Sotres-Bayon, Cain, & LeDoux, 2006), with some evidence to suggest increases in activation over the course of extinction learning (Guhn et al., 2012). The vmPFC has been shown to be active not only during extinction learning, but also when extinction recall is tested at follow-up (Milad et al., 2007; Phelps et al., 2004). Taken together with the results of animal studies, the vmPFC is thought to store new associations and use this information to appropriately inhibit the amygdala (Graham & Milad, 2011; Rauch et al., 2006).

Fear extinction studies in humans have also implicated the hippocampus. The hippocampus is involved in formation of new memories and may be necessary for the acquisition of complex and highly contextualized associations during fear extinction (R. Phillips & LeDoux, 1992). The integration of contextual information during fear conditioning via the hippocampus has been hypothesized as a mechanism that facilitates the return of fear following successful extinction, by linking fear extinction

learning to a specific context (Milad, Rosenbaum, & Simon, 2014). Hippocampus activation has also been observed during extinction recall (Milad et al., 2007).

Neural bases of fear conditioning in anxious adults. The same regions implicated in animal models and neuroimaging in healthy adults also demonstrate differential activation in anxious relative to non-anxious individuals. The most research in the neural bases of fear extinction in anxiety has been done in post-traumatic stress disorder (PTSD). Functional neuroimaging has revealed that relative to non-anxious controls, individuals with PTSD exhibit greater amygdala and dorsal ACC, as well as less vmPFC activation during fear extinction (Bremner et al., 2005; Milad et al., 2009) and extinction recall (Milad et al., 2009; Rougemont-Bücking et al., 2011). Furthermore, increased vmPFC activation in response to fearful facial expressions has been observed following successful exposure therapy for PTSD (Felmingham et al., 2007). Greater amygdala and less vmPFC activation during fear extinction has also been associated with higher levels of trait anxiety in non-disordered adults (Barrett & Armony, 2009).

Although the neural substrates of fear extinction have not been examined in public speaking anxiety specifically, several studies have examined fear extinction using neuroimaging in generalized social phobia. Results of these studies have been inconsistent, with several finding no differences in brain activation between socially anxious individuals and healthy controls during fear extinction (Schneider et al., 1999; Veit et al., 2002), and one study reporting an *inverse* correlation between amygdala activation during fear extinction and levels of social anxiety (Pejic et al., 2013).

However, prior research in social phobia has exclusively relied on social stimuli such as neutral faces as the conditioned stimuli (Hermann et al., 2002; Lissek et al., 2008; Pejic et al., 2013; Schneider et al., 1999; Veit et al., 2002). Use of social stimuli in social phobia introduces the potential confound of pre-existing differences in reactivity to the stimuli between cases and controls prior to any experimental manipulation. In contrast, more neutral stimuli such as lights, colors, or tones, have been used in fear conditioning studies in other anxiety disorders (Lissek et al., 2005). Furthermore, because of the need for a comparison condition in fMRI analysis, fear conditioning paradigms used in fMRI research typically make use of discriminant conditioning tasks. Use of social stimuli such as faces as conditioned stimuli may therefore make differential activation (i.e., CS+ versus CS-) more difficult to observe, particularly in structures such as the amygdala that are known to be hyper-responsive to faces in socially anxious individuals (Cooney, Atlas, Joormann, Eugène, & Gotlib, 2006).

The present study therefore examines the relationship between public speaking anxiety and the neural bases of fear extinction learning using non-social conditioned stimuli. Furthermore, the relationship between neural bases of extinction learning and exposure success is examined, which, although theoretically important, has not been investigated to date.

Hypotheses

Three main hypotheses guided the current work. Based on prior research in individuals with anxiety disorders and non-disordered adults with high trait anxiety

(Milad et al., 2009; Sehlmeyer et al., 2011), the first hypothesis was that public speaking anxiety severity would be associated with amygdala and vmPFC activation during fear extinction such that those with greater severity would show greater amygdala and less vmPFC activation. Next, based on the amygdala's role in the representation of fear (Maren & Quirk, 2004), the second hypothesis was that less amygdala activation during extinction would predict greater short-term anxiety reduction (i.e., anxiety reduction across several exposures). Finally, based on the role of the vmPFC in consolidating and integrating fear extinction learning (R. McNally, 2007; Milad et al., 2007), the third hypothesis was that greater vmPFC activation during extinction would predict greater reduction of anxiety two weeks following the exposure session.

Methods

Participants

The University of California San Diego and San Diego State University Human Research Protections Programs approved this study. After providing written informed consent, 39 participants who self-identified as having high public speaking anxiety were screened by self-report questionnaires and a semi-structured diagnostic interview (Lecrubier et al., 1997). Of these, 27 were eligible, and 24 completed all study procedures. Eligible participants scored at least 20 on the Personal Report of Confidence as a Speaker (PRCS) questionnaire, representing the 75th percentile (G. Phillips, Jones, Rieger, & Snell, 1997). Participants were excluded for psychotropic

medication use in the past four weeks, prior experience with exposure therapy, history of bipolar disorder, psychotic disorder, or drug or alcohol dependence. Participants were also exlcuded for current major depressive disorder of greater than moderate severity (defined as a Patient Health Questionnaire score of > 14) or for clinically significant suicidal or homicidal ideation. In addition, participants met fMRI safety and eligibility criteria: no non-removable ferrous metal, no neurological conditions, no history of loss of consciousness greater than five minutes, no pregnancy, no claustrophobia, and no medical conditions that would preclude lying still in the scanner for approximately one hour.

Measures

Measures of public speaking anxiety, social anxiety, overall anxiety, disability, and depression were acquired. Each is described below. In addition to those instruments, several other measures were used during the course of the study. Diagnoses were established using a semi-structured interview (Mini International Neuropsychiatric Interview; Lecrubier et al., 1997) to determine eligibility and describe the sample. Although this interview was developed for DSM-IV, additional questions were asked so that social anxiety disorder diagnostic status could also be determined based on DSM-5 criteria. To measure fear conditioning, participants were asked to rate their valence and arousal to the CS+ and CS- periodically throughout the fear conditioning task using a 5-point manikin scale (Bradley & Lang, 1994) as in Sehlmeyer et al. (2011). In addition, participants were asked to rate their level of anxious distress to each picture using a 0 to 100 subjective unit of distress scale

(SUDS). During the exposures, ongoing anxiety was also assessed using the SUDS, with participants rating their distress each minute.

Public speaking anxiety. The PRCS (Paul, 1966) was used to assess public speaking anxiety and as the primary outcome measure. This instrument is widely used, has shown good psychometric properties, has published norms, and has previously been used in treatment studies (G. Phillips et al., 1997).

Social anxiety. To further describe the sample, two measures of general social anxiety severity with good psychometric properties were also acquired. The Brief Fear of Negative Evaluation (BFNE) questionnaire (Leary, 1983) is a self-report measure that assesses concern about negative evaluation, a key construct thought to underlie social phobia. The Liebowitz Social Anxiety Scale (LSAS; Heimberg et al., 1999) is a clinician-administered measure that assesses anxiety and avoidance of 24 social and performance situations. Both measures are widely used to assess social anxiety.

Overall anxiety. The Overall Anxiety Severity and Impairment Scale (OASIS; Norman, Cissell, Means-Christensen, & Stein, 2006) was used to describe the overall anxiety pathology of the sample. Furthermore, in order to measure state anxiety at each study visit, the State version of the State-Trait Anxiety Inventory was used (Spielberger, Gorsuch, & Lushene, 1970).

Disability. The Sheehan Disability Scale (SDS; Sheehan, 1983) was also used to assess the degree of disability of the sample. The SDS is a widely used measure with good psychometric properties (Leon, Olfson, Portera, Farber, & Sheehan, 1997).

Depression. Because depression so often co-occurs with anxiety (Kessler et al., 2005a), depression symptoms were assessed with the Patient Health Questionnaire (PHQ-9; Kroenke, Spitzer, & Williams, 2001) in order to confirm eligibility, describe the sample, and use as a covariate to test the robustness of the results. The PHQ-9 is widely used, has published clinical cut-offs, and good psychometric properties (Gilbody, Richards, Brealey, & Hewitt, 2007; Kroenke et al., 2001; Löwe, Unützer, Callahan, Perkins, & Kroenke, 2004).

Task

The fear conditioning task was based closely on the task successfully used by Sehlmeyer et al. (2011) to uncover neural bases of fear conditioning associated with trait anxiety. The stimuli consisted of two neutral, non-social, abstract images as conditioned stimuli (CS), presented for two seconds at a time. The image assigned as the CS+ (paired with the unconditioned stimulus (US) during fear acquisition) and the CS- (never paired with the US) was counter-balanced across participants. The US was a loud scream beginning one second after CS+ onset and lasting 800ms (Glenn et al., 2012). In the 9-15 seconds between CS image presentations, participants were engaged in a continuous performance task requiring a right or left button press in response to right or left facing arrows. This served to increase engagement and attention in the inter-trial interval.

The task consisted of three phases: a brief familiarization period, fear acquisition, and fear extinction. First, the *familiarization phase* (2.5 minutes) involved five presentations of each CS with no instances of the US. This served to allow

familiarization to the task and scanner environment. Next, the *acquisition phase* was broken into two runs of 8.5 minutes each. Each run consisted of 15 presentations of the CS- and 20 presentations of the CS+: five with (CS+ paired) and 15 without (CS+ unpaired) the US. This follows Sehlmeyer et al., (2011) and allows for an equal number of trials to be included in the analysis (the CS+ paired trials are excluded from analysis so as to not confound processing of the CS+ with reactivity to the US). Finally, the *extinction phase* (12 minutes) involved 25 presentations of each CS with no instances of the US. Participants rated their valence, arousal, and anxiety to each CS at four times during the task: after familiarization, halfway through acquisition, after acquisition, and after extinction. Trials were presented in a fixed, pseudo-randomized order, constrained so that no more than two identical trials occurred in a row.

Procedure

The study involved three-in person visits and a follow-up phone call. Prior to the study visits, the first contact with participants was a brief telephone screen in which the study was described and likely eligibility established via an fMRI safety screen and administration of the PRCS by phone. Eligible participants based on the phone screen were invited for a diagnostic interview (visit 1). At this visit written informed consent was obtained, and participants completed the diagnostic interview (MINI) and clinician-administered LSAS, as well as self-report measures (BFNE, STAI-State, PHQ-9, PRCS, OASIS, SDS).

Eligible participants based on the initial visit then completed an fMRI visit at the UCSD Center for fMRI (visit 2). Prior to the scan, participants received training on the task using different, though similar, CSs from the task itself. Participants were also exposed to an example of the US. The scan itself involved the fear conditioning task (35 minutes) as well as a high-resolution anatomical scan (8.5 minutes).

The third visit took place on a separate day, and consisted of a massed exposure protocol preceded by a presentation of the rationale for exposure therapy by a therapist (TMB). The protocol was based closely on a previously used manual (Vasey et al., 2012). Participants completed a series of four public speaking exposures lasting five minutes each. For each speech participants were given two topics and asked to speak about one or both topics. Prior to each speech participants received two minutes to prepare. Each speech was presented to an active video camera, the therapist, and two confederates. Participants were asked to stay in front of the camera for the full five minutes, and reminded of this by therapist prompts (e.g., "you can repeat things you've already said"). Confederates and the therapist maintained a neutral facial expression, provided no verbal or non-verbal feedback (e.g., saying "mm-hmm," nodding, smiling) and made frequent eye contact with the participant. SUDS ratings were recorded at the beginning and end of the preparation period and every minute during the speech. Participants had one minute to rest between speeches, and the therapist provided a brief reminder of the rationale for exposure at the end of each rest interval.

Two weeks after the exposure visit, participants were contacted by telephone and completed the PRCS, LSAS, and SDS in order to examine longer-term effects of exposure on public speaking anxiety, general social anxiety and avoidance, and overall disability. Participants were asked about any exposures to public speaking since the exposure visit (one person reported a group presentation during the follow up period). Participants were instructed to refrain from engaging in therapy or psychotropic medications during the follow-up period; all participants reported compliance with this instruction.

Image Acquisition

The fear conditioning tasks consisted of four runs: run one (familiarization), lasted 2 minutes 33 seconds, runs two and three (acquisition) lasted 8 minutes 24 seconds each, and run four (extinction) lasted 11 minutes 54 seconds. BOLD signal was acquired, using a Signa EXCITE 3.0 Tesla-GE scanner (T2*-weighted echo planar imaging, TR = 1500 ms, TE = 32 ms, FOV = 240 × 240 mm^3, thirty 3 mm axial slices with a 1 mm gap). For anatomical reference, a high resolution T1-weighted image (SPGR, TR = 8 ms, TE = 3 ms, FOV = 256 × 256 mm^3, flip angle = 12°, 172 sagittally acquired slices with 1 mm thickness) was obtained during the same session.

Statistical Analysis

fMRI image processing and analysis of individual participant data was conducted using Analysis of Functional NeuroImages (AFNI) software (Cox, 1996). Specific hypotheses were then tested with group-level statistics implemented in the R

statistical package (http://cran.r-project.org). Statistical analysis of non-brain imaging data was done using SPSS.

Individual-level image processing. Image processing involved first adjusting voxel time series for non-simultaneous slice acquisition and correcting for motion. Functional images were aligned to the anatomical images through a co-registration algorithm (Saad et al., 2009), and images were spatially smoothed to a 6 mm Gaussian filter. Finally, data were aligned to a standard template (Talairach & Tournoux, 1988). The AFNI program *3dToutcount* was used to determine the number of voxels classified as outliers at each time point. Any time point with greater than two standard deviations more outlier voxels than the participant's mean was excluded from analysis. Data quality was ensured through visual inspection as well as computation of motion parameters (>6mm motion was grounds for exclusion) and number of outlier time points (>5% was grounds for exclusion). No participants were excluded for poor data quality (maximum motion was 3.4mm, maximum outlier percentage was 4.1%). Visual inspection confirmed no gross anatomical abnormalities and adequate alignment between functional and anatomical images for all subjects.

Individual-level task analysis. Individual time series data were then analyzed with AFNI's *3dDeconvolve* program to generate acquisition and extinction related activation at each voxel. This program regresses the expected activation time series for brain regions responding to a stimulus (e.g., the CS+) on the observed time series in each voxel.

The result of this analysis yielded activation for each participant to the CS+ and CS- during acquisition and extinction, with each task component broken into early and late halves. In other words, this is a two (stimulus: CS+, CS-) by four (time: early acquisition, late acquisition, early extinction, late extinction) design. Although an overall interaction effect was expected in both amygdala and mPFC across all participants, the contrast between activation to the CS+ versus CS- during early and late extinction, calculated at each voxel for each participant, were primarily used.

Group analysis overview. Consistent with the aims of this project, analyses focused on individual differences in extinction-related brain activation. Future research should examine the role of individual differences in the neural bases of fear acquisition. T-tests were used to identify main effects of the task. Regression analyses, specifically robust regressions (Huber, 1973) were used for all individual difference analyses. Robust regression is more robust to the presence of outliers; because it is impractical to examine distributions of every voxel in the brain robust regressions are recommended for fMRI analysis (Wager, Keller, Lacey, & Jonides, 2005). Analyses were conducted voxel-wise within the amygdala and mPFC regions of interest, with follow-up analyses voxel-wise across the whole brain.

Hypothesis one: Anxiety severity. The first hypothesis was that public speaking anxiety severity would be associated with amygdala and mPFC activation during fear extinction such that those with greater anxiety would show greater amygdala and less ventromedial prefrontal activation. To test this hypothesis, regressions with early and late extinction activation as the dependent variable and

public speaking anxiety (PRCS) as the independent variable were conducted within the a priori regions of interest: mPFC and amygdala.

In addition, exploratory analyses were conducted across the whole brain in an attempt to identify relationships between PRCS and activation in additional, non-hypothesized regions during extinction. Relationships with overall anxiety severity (OASIS) were also examined across the whole brain, in order to explore the relationship between neural bases of fear extinction and broader anxiety pathology.

Hypothesis two: Short-term exposure outcomes. The second hypothesis was that less amygdala activation during extinction would predict greater short-term anxiety reduction (i.e., between-exposure habituation). To test this hypothesis, regressions with early and late extinction activation as the dependent variable and change in peak SUDS ratings from the first to the last exposure as the independent variable were conducted within the amygdala. Peak SUDS rating from the first speech was included as a covariate in this analysis. Exploratory analyses conducted across the whole brain searched for non-hypothesized regions associated with between-exposure habituation, controlling for first speech SUDS.

Hypothesis three: Longer-term exposure outcomes. The third hypothesis was that greater vmPFC activation during fear extinction would predict greater reduction of anxiety two weeks following the exposure sessions. To test this hypothesis regressions with early and late extinction activation as the dependent variable and change in public speaking anxiety (PRCS) from the diagnostic visit to

two week follow-up as the independent variable were conducted within the mPFC. Baseline PRCS was included as a covariate in this analysis.

Exploratory analyses conducted across the whole brain searched for non-hypothesized regions associated with longer-term public speaking anxiety reduction (change in PRCS), controlling for baseline PRCS. In addition, relationships with change in overall social anxiety severity (LSAS) from the diagnostic visit to two week follow-up were examined across the whole brain, in order to explore the relationship between neural bases of fear extinction and generalization of exposure learning.

Additional individual difference analyses. Several additional, exploratory analyses were conducted across the whole brain to better understand individual differences in neural activation. First, to better understand the main effect of the task, robust regressions were conducted with activation to CS+ relative to CS- during early and late acquisition and extinction as the dependent variables, and valence ratings of CS+ relative to CS- as the independent variable. Second, to examine whether results of analyses involving general social anxiety (LSAS) were driven by public speaking anxiety items, these analyses were re-run with these items (items number six, 16, and 20) removed from the total score. Finally, all individual difference analyses were run both with and without depressive symptoms (PHQ-9) as a covariate to confirm the pattern of results.

Correction for multiple comparisons. Type I error was controlled through joint statistical ($p<.01$) and volume thresholds as determined by Monte Carlo simulations (n=10000) using AFNI's *3dClustSim* program such that the cluster-wise

α=.05. The result of these simulations indicates that for analyses in the amygdala, clusters of significant ($p<.01$) voxels must be at least 192 μL. In the mPFC, clusters of significant ($p<.01$) voxels must be at least 384 μL. For exploratory analyses across the entire brain, clusters of significant ($p<.01$) voxels must be at least 768 μL.

Results

Participant demographic and clinical characteristics are summarized in Tables 1 and 2. Ten participants (42%) met DSM-IV criteria for generalized social anxiety

Table 1. Demographics and Baseline Self-Report Measures

Measure	Mean (SD)	Range
Age	21.9 (4.4)	18 – 35
Gender (% female)	71%	-
Ethnicity (%)		
Caucasian	25%	-
Asian	33%	-
Latino	29%	-
Other	13%	-
PRCS	23.1 (2.7)	18 – 29
LSAS total	51.2 (19.4)	17 – 94
Anxiety subscale	28.2 (10.2)	11 – 51
Avoidance subscale	23.0 (9.8)	6 – 45
OASIS	6.0 (3.0)	1 – 13
BFNE	42.5 (8.7)	28 – 59
STAI-S	37.9 (9.2)	21 – 59
PHQ-9	3.8 (3.8)	0 – 12
SDS	4.3 (5.4)	0 – 17

Note. SD = standard deviation, PRCS = Personal Report of Confidence as a Speaker, LSAS = Liebowitz Social Anxiety Scale, OASIS = Overall Anxiety Severity and Impairment Scale, BFNE = Brief Fear of Negative Evaluation, STAI-S = State Trait Anxiety Inventory – State, PHQ-9 = Patient Health Questionnaire – 9 item version, SDS = Sheehan Disability Scale.

disorder, and an additional seven (29%) met DSM-IV criteria for non-generalized social anxiety disorder. Six (25%) reported a past major depressive episode; no participants met DSM-IV criteria for a current depressive episode, and none had PHQ-9 scores indicating greater than moderate depressive symptoms (range: 0-12). Self-report measures of social anxiety (LSAS), overall anxiety (OASIS), depression (PHQ-9), and disability (SDS) were all highly correlated at baseline ($r=.63$-$.84$, $p<.001$). Public speaking anxiety (PRCS) was not correlated with these measures ($p>.7$).

Table 2. Clinical Characteristics of Participants

Disorder	Diagnosed N (%)	Primary N (%)
Social Anxiety Disorder		
Generalized	10 (42)	9 (38)
Non-generalized	7 (29)	7 (29)
Generalized Anxiety Disorder	1 (4)	1 (4)
Panic Disorder	0 (0)	0 (0)
Agoraphobia	1 (4)	0 (0)
PTSD	0 (0)	0 (0)
OCD	1 (4)	0 (0)
Alcohol Abuse	1 (4)	0 (0)
Substance Abuse	0 (0)	0 (0)
Major Depressive Disorder		
Current	0 (0)	0 (0)
Past	6 (25)	0 (0)
Anorexia	0 (0)	0 (0)
Bulimia	0 (0)	0 (0)

Note. Diagnoses based on DSM-IV. Six of seven participants who met DSM-IV criteria for non-generalized social anxiety disorder also met for DSM-5 performance only specifier. One participant's symptoms were not generalized but were also not limited to performance situations (i.e., performance and dating). All who met social anxiety disorder criteria met for both DSM-IV and DSM-5. Lifetime psychosis, alcohol or substance dependence, and bipolar disorder were excluded; no participants met criteria for any of these conditions. Seven participants did not meet criteria for any diagnoses and therefore had no primary diagnosis. PTSD = Post-Traumatic Stress Disorder, OCD = Obsessive-Compulsive Disorder.

Exploratory one-way analysis of variance were used to investigate differences on self-report measures by social anxiety diagnostic status (generalized, non-generalized, or no diagnosis). Individuals with generalized social anxiety disorder had higher scores on disability (SDS), social anxiety (LSAS), and depression (PHQ-9) than non-generalized and non-diagnosed individuals ($p<.05$, Tukey corrected for multiple comparisons), who did not differ from each other on these measures ($p>.7$). Overall anxiety severity (OASIS) was greater in diagnosed than non-diagnosed individuals ($p<.05$, Tukey corrected for multiple comparisons); individuals diagnosed with generalized social anxiety disorder did not differ from those diagnosed with non-generalized social anxiety disorder ($p>.1$). The primary outcome measure of public speaking anxiety (PRCS) did not differ between the three diagnostic groups ($p>.7$).

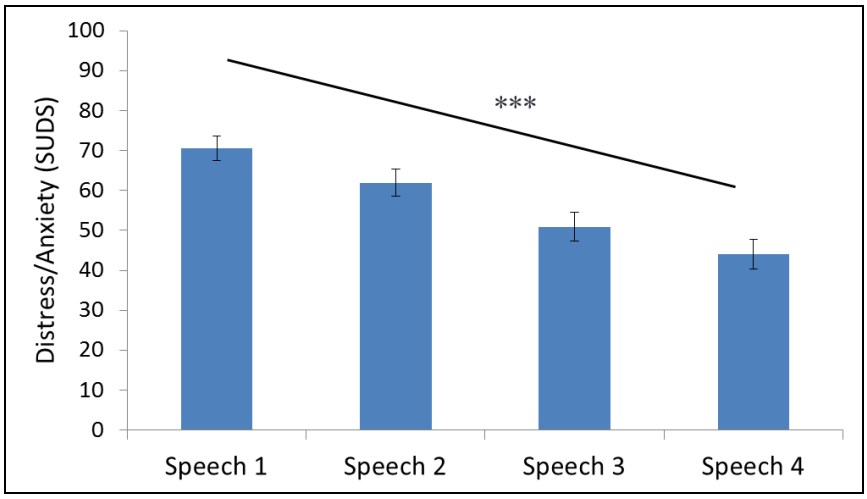

Figure 2. Average peak rating for each speech. *** indicates significant linear main effect of speech, $p<.001$

Main Effects of Massed Exposure

Short-term effects of the massed speech exposure paradigm were assessed by 0-100 point SUDS ratings. Figure 2 shows the average *peak* SUDS rating for each speech. A one-way, repeated measures ANOVA conducted on the peak ratings showed a significant main effect of time on SUDS ratings ($F(3,69)=43.3$, $p<.001$, partial $\eta^2=.65$), with follow-up contrasts confirming a linear effect ($F(1,23)=56.5$, $p<.001$, partial $\eta^2=.71$; quadratic and cubic effects ns).

Longer-term effects of the exposure session on public speaking anxiety (PCRS), social anxiety (LSAS) and disability (SDS) were assessed at 2-week follow-up (Table 3). Paired t-tests revealed significant changes over time on the PRCS ($t(23)=3.83$, $p=.001$, $d=.78$) and LSAS ($t(23)=2.86$, $p=.009$, $d=.58$), but not the SDS ($t(23)=-.53$, $p=.600$, $d=.11$). Changes in LSAS and PRCS did not differ by social anxiety diagnosis (generalized, non-generalized, or no diagnosis; $p>.3$), and were not correlated with baseline measures of anxiety (LSAS, OASIS, PRCS), depression (PHQ-9), or disability (SDS; all p's$>.06$).

Table 3. Change from Baseline to Follow-up on Self-Report Measures

	Baseline Mean (SD)	Follow-up Mean (SD)	Paired t-test t-value (p-value)
PRCS	23.1 (2.7)	20.2 (4.6)	3.83 (.001)
LSAS total	51.2 (19.4)	43.7 (18.7)	2.86 (.009)
Anxiety subscale	28.2 (10.2)	23.8 (9.7)	3.17 (.004)
Avoidance subscale	23.0 (9.8)	19.8 (10.1)	2.27 (.033)
SDS	4.3 (5.4)	4.9 (4.8)	-0.53 (.600)

Note. SD = standard deviation, PRCS = Personal Report of Confidence as a Speaker, LSAS = Liebowitz Social Anxiety Scale, SDS = Sheehan Disability Scale.

Self-Report Ratings of Fear Conditioning

Fear acquisition and extinction were assessed by valence, arousal, and anxiety ratings for each CS after each of the four fMRI runs: familiarization, mid-acquisition, post-acquisition, and post-extinction. Mid-acquisition and post-acquisition ratings were averaged resulting in a two (stimulus: CS+, CS-) by three (condition: familiarization, acquisition, extinction) design. Familiarization ratings for one subject were not collected, therefore N=23 for these analyses. Significant fear acquisition and extinction was demonstrated by all three ratings as shown in Figure 3. There were no

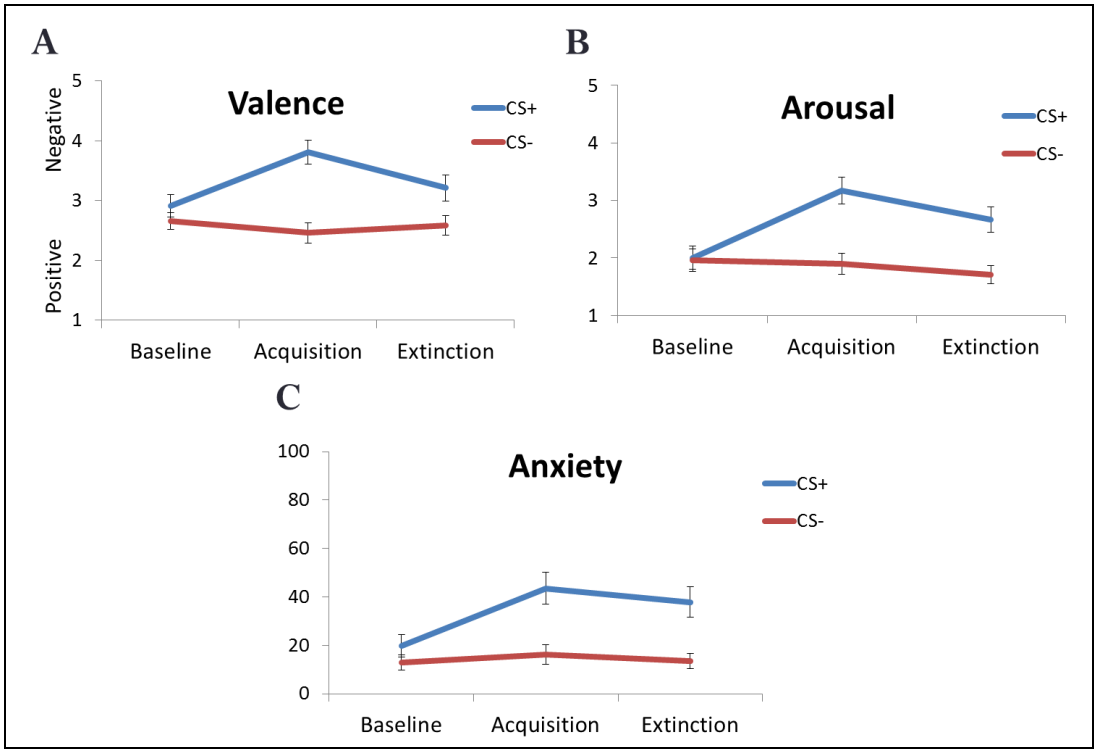

Figure 3. Self-report ratings of (A) valence, (B) arousal, and (C) anxiety, collected after each phase of the fear conditioning task. Significant condition by task interaction present for all three ratings, $p<.001$.

significant correlations between any differential (i.e., CS+ versus CS-) ratings for either acquisition or extinction and any baseline anxiety measures (OASIS, LSAS, PRCS; all p's >.09).

Valence. Valence ratings showed a significant main effect of stimulus ($F(1,22)=19.97$, $p<.001$, partial $\eta^2=.49$), with the CS+ rated more negatively than the CS-. There was also a main effect of condition on valence ratings ($F(2,44)=3.59$, $p=.036$, partial $\eta^2=.14$); follow-up contrasts indicated significantly more negative ratings during acquisition than during familiarization ($t(22)=2.48$, $p=.02$, partial $\eta^2=.22$), followed by a significant decrease in negative ratings from acquisition to extinction ($t(22)=2.50$, $p=.02$, partial $\eta^2=.22$). Finally, there was a stimulus by condition interaction ($F(2,44)=13.37$, $p<.001$, partial $\eta^2=.38$) such that there was an increase in negative ratings from familiarization to acquisition for CS+ more than for CS- ($t(22)=5.16$, $p<.001$, partial $\eta^2=.55$), and a decrease in negative ratings from acquisition to extinction for CS+ more than for CS- ($t(22)=3.96$, $p=.001$, partial $\eta^2=.42$) (Figure 3A).

Arousal. Arousal ratings demonstrated the same pattern. There was a significant main effect of stimulus ($F(1,22)=25.16$, $p<.001$, partial $\eta^2=.53$), with greater arousal to the CS+ than the CS-. There was also a main effect of condition on arousal ratings ($F(2,44)=5.86$, $p=.006$, partial $\eta^2=.21$); follow-up contrasts indicated significantly greater arousal during acquisition than familiarization ($t(22)=3.29$, $p=.003$, partial $\eta^2=.33$), followed by a significant reduction in arousal from acquisition to extinction ($t(22)=2.61$, $p=.02$, partial $\eta^2=.24$). Finally, there was a stimulus by

condition interaction ($F(2,44)=14.98$, $p<.001$, partial $\eta^2=.41$). Follow-up contrasts indicated that arousal ratings increased from familiarization to acquisition for CS+ more than CS- ($t(22)=4.54$, $p<.001$, partial $\eta^2=.48$) and decreased from acquisition to extinction for CS+ more than CS- ($t(22)=2.07$, $p=.05$, partial $\eta^2=.16$) (Figure 3B).

Anxiety. Anxiety ratings demonstrated the same pattern. There was a significant main effect of stimulus ($F(1,22)=28.93$, $p<.001$, partial $\eta^2=.57$), with greater anxiety to the CS+ than the CS-. There was also a main effect of condition on anxiety ratings ($F(2,44)=10.87$, $p<.001$, partial $\eta^2=.33$); follow-up contrasts indicated significantly elevated anxiety for acquisition relative to familiarization ($t(22)=3.83$, $p=.001$, partial $\eta^2=.40$), and a significant reduction in anxiety for extinction relative to acquisition ($t(22)=2.59$, $p=.02$, partial $\eta^2=.23$). Finally, there was a stimulus by condition interaction ($F(2,44)=10.84$, $p<.001$, partial $\eta^2=.33$). Follow-up contrasts indicated that anxiety ratings increased from familiarization to acquisition for CS+ more than for CS- ($t(22)=4.10$, $p<.001$, partial $\eta^2=.43$), but CS+ and CS- ratings decreased equivalently from acquisition to extinction ($p=.12$) (Figure 3C).

Main Effect of the Fear Conditioning Task on Neural Activation

One-sample t-tests were conducted on a voxel-wise basis to examine main effects of the fear conditioning task (i.e., activation to CS+ versus CS-) during early and late components of fear acquisition and extinction. The results are summarized in Table 4. Briefly, while there were few differences between CS+ and CS- during early acquisition, during late acquisition activation in right insula, dorsal ACC, and right

Table 4. Fear Conditioning Task Main Effects on Brain Activation

Brain Region	**BA**	**Vol**	**X**	**Y**	**Z**	**Peak t**
Early Acquisition						
Medial precuneus	7	768	-2	-53	40	3.43
Late Acquisition						
R inferior parietal lobule / supramarginal gyrus	40	2112	62	-45	24	2.88
Medial precuneus / paracentral lobule	31; 7	1664	2	-29	44	4.13
L inferior parietal lobule / supramarginal gyrus	40	1408	-58	-45	36	3.97
Dorsal ACC	32	1024	2	11	40	3.06
R insula / putamen	13	896	38	-13	0	3.44
Dorsomedial PFC*	6	448	10	7	60	3.58
Early Extinction						
R inferior parietal lobule / supramarginal gyrus	40	1984	62	-45	28	5.11
R inferior frontal gyrus	44	1600	54	15	4	2.94
Dorsal mid-cingulate	23	1344	2	-25	28	2.95
R inferior parietal lobule	40	896	46	-29	44	-2.95
R middle temporal gyrus	22; 21	768	50	-29	0	3.78
Late Extinction						
Dorsal mid-cingulate	23	1024	2	-21	28	3.80

Note. *indicates small volume corrected, coordinates are in Talairach space. BA = Brodmann area, Vol = volume, R = right, L = left, PFC = prefrontal cortex, ACC = anterior cingulate cortex

inferior parietal lobule emerged. During extinction, activation in the right inferior parietal lobule remained and activation in the right inferior frontal gyrus emerged; these regions were no longer activated in late extinction.

To better understand the task effects, robust regression analyses were conducted on a voxel-wise basis using self-reported valence ratings (i.e., CS+ versus CS-) as predictors of activation (CS+ versus CS-) in each component of the task. The results are summarized in Tables 5 and 6. Briefly, during both acquisition (Table 5) and extinction (Table 6), individuals who rated the CS+ more negatively than the

Table 5. Acquisition-Related Activations Associated with Task Ratings

Brain Region	BA	Vol	X	Y	Z	Peak t
Early Acquisition						
Periaqueductal grey		1216	6	-25	-24	3.16
R postcentral gyrus	3; 43	1216	54	-17	16	3.12
L cerebellum		832	-30	-53	-28	3.20
R uncus / parahippocampal gyrus	28	768	30	-9	-28	2.91
L postcentral gyrus	2; 3	768	-42	-17	40	4.49
R amygdala / parahippocampal gyrus*	34	448	18	-5	-16	3.38
Late Acquisition						
R precuneus / superior parietal lobule	7	896	30	-69	44	-4.22
R dorsal PFC	6; 8	896	38	15	52	-3.23
L supramarginal / angular gyrus	39	768	-38	-57	32	-4.92

Note. *indicates small volume corrected, coordinates are in Talairach space. BA = Brodmann area, Vol = volume, R = right, L = left, PFC = prefrontal cortex

CS- also showed greater activation to CS+ relative to CS- in the periaqueductal grey and amygdala. During extinction, individuals who rated the CS+ more negatively than the CS- showed greater activation to CS+ relative to CS- in bilateral anterior insula, OFC, dorsolateral PFC, dorsomedial PFC, and dorsal ACC (Table 6).

Hypothesis One: Anxiety Severity

The first hypothesis was that anxiety severity would be associated with amygdala and mPFC activation during fear extinction such that those with greater anxiety would show greater amygdala and less mPFC activation. Relationships between public speaking anxiety as measured by the PRCS and activation in either amygdala or medial PFC were not found. Whole brain analysis uncovered no regions where public speaking anxiety was associated with brain activation during early or late extinction.

Table 6. Extinction-Related Activations Associated with Task Ratings

Brain Region	BA	Vol	X	Y	Z	Peak t
Early Extinction						
L dlPFC / anterior insula / IFG / OFC / caudate	9; 8; 13; 44; 10	19200	-30	43	-8	4.36
Dorsal ACC / dmPFC	24; 32; 6	12864	2	3	44	4.76
R anterior insula	13	5184	46	15	0	4.30
R OFC / dlPFC	10; 9; 46	3776	26	51	32	4.08
R temporal pole	38	3264	26	3	-28	3.09
L supramarginal gyrus / inferior parietal lobule	40	2496	-62	-41	24	4.16
Medial lingual gyrus / cerebellum	18	2240	6	-77	-16	3.07
R middle / superior temporal gyrus	21; 22	2112	66	-21	0	3.51
L uncus	20	1600	-26	-1	-36	3.48
L middle / superior temporal gyrus	22; 39	1408	-58	-53	8	4.14
R precentral / postcentral gyrus	3; 4	1344	38	-29	60	2.91
L supramarginal gyrus / inferior parietal lobule	40	1216	-34	-49	36	4.69
R posterior insula	13	1152	62	-37	20	5.18
R IFG	47	1088	22	15	-16	4.87
R precuneus	7	1024	10	-65	28	5.58
Periaqueductal grey		960	6	-21	-20	3.61
Medial cuneus / lingual gyrus	17	896	10	-97	8	4.76
R superior temporal / supramarginal gyrus	39; 22	896	42	-49	24	4.09
L precuneus	7	896	-10	-49	60	4.83
L IFG	47	832	-22	19	-24	3.07
L lingual gyrus / cerebellum	19	768	-10	-61	-4	3.48
Dorsomedial PFC*	6	640	2	7	44	4.11
Medial OFC*	10	384	14	43	12	4.56
L amygdala*		320	-22	-1	-20	3.75
Late Extinction						
dmPFC	6; 8	3584	2	19	56	4.99
L anterior insula / IFG	13; 45	2432	-42	19	8	4.76
R anterior insula / IFG	13; 47	1600	22	11	-16	4.38
L dlPFC	9; 10	1536	-30	55	20	3.08
dmPFC	9; 10	1408	2	51	20	4.85
R dorsal PFC	6	1280	18	7	64	6.55
dmPFC*	9	448	-2	43	28	4.08

Note. *indicates small volume corrected, coordinates are in Talairach space. BA = Brodmann area, Vol = volume, R = right, L = left, PFC = prefrontal cortex, dlPFC = dorsolateral prefrontal cortex, dmPFC = dorsomedial prefrontal cortex, OFC = orbitofrontal cortex, ACC = anterior cingulate cortex, IFG = inferior frontal gyrus.

Table 7. Extinction-Related Activations Associated with Overall Anxiety Severity

Brain Region	BA	Vol	X	Y	Z	Peak t
Early Extinction						
None						
Late Extinction						
Dorsomedial PFC	6; 8	960	-2	31	56	3.27
L dorsolateral PFC	9; 10	768	-30	47	28	3.62
L inferior frontal gyrus	47	896	-42	27	0	3.07
L inferior frontal gyrus	45	832	-50	23	20	3.82

Note. Coordinates are in Talairach space. BA = Brodmann area, Vol = volume, L = left, PFC = prefrontal cortex

Follow-up analyses to examine overall anxiety severity as measured by the OASIS found that individuals with higher anxiety showed *greater* activation in dorsomedial PFC to the CS+ relative to the CS- in the second half of extinction. Greater anxiety was similarly associated with activation in left dorsolateral PFC and left inferior frontal gyrus (Table 7).

Table 8. Extinction-Related Activations Associated with Anxiety Reduction during Exposure

Brain Region	BA	Vol	X	Y	Z	Peak t
Early Extinction						
R precuneus	7	1088	22	-65	32	-2.92
L postcentral gyrus	2	896	-46	-29	28	-2.84
R middle occipital / middle temporal gyrus	19; 39	832	34	-77	4	-3.95
Late Extinction						
None						

Note. Coordinates are in Talairach space. BA = Brodmann area, Vol = volume, L = left, R = right.

Hypothesis Two: Short-Term Exposure Outcomes

The second hypothesis was that less amygdala activation during extinction would predict greater short-term anxiety reduction (i.e., change in anxiety from first to

last speech measured by SUDS). However, amygdala activation during extinction was unrelated to anxiety reduction across the exposure session. Instead, individuals who showed less activation to the CS+ relative to the CS- during extinction in right precuneus, left postcentral gyrus, and right middle occipital gyrus reported greater reduction in anxiety from the first to the last speech (Table 8).

Hypothesis Three: Longer-Term Exposure Outcomes

The third hypothesis was that greater mPFC activation during fear extinction would predict greater reduction of public speaking anxiety (PRCS) two weeks following the exposure sessions. Indeed, individuals with greater activation in the ventral ACC in the first half of extinction had greater reduction in public speaking anxiety from baseline to two-week follow-up (Figure 4). In addition, individuals with

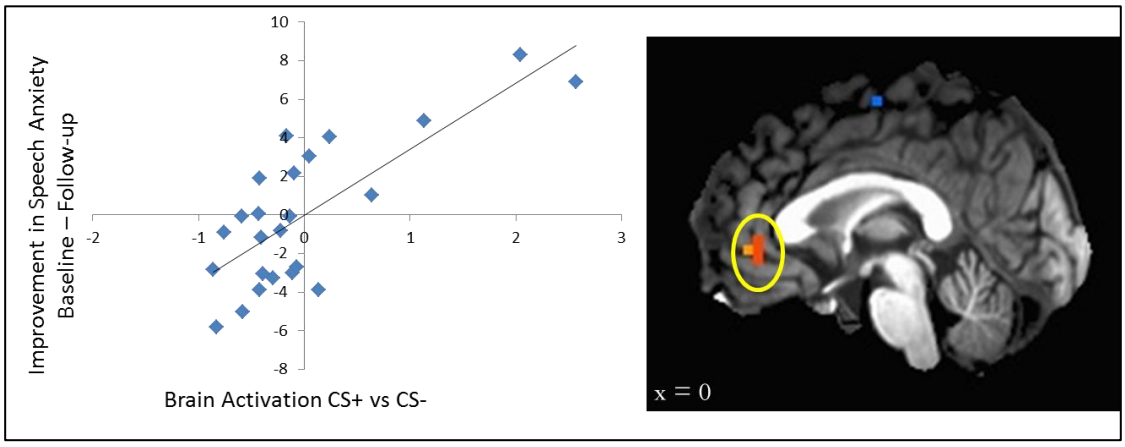

Figure 4. Region within medial prefrontal cortex associated with reduction in speech anxiety controlling for baseline speech anxiety. Red color indicates positive association, blue indicates inverse association. Graph indicates relationship between average activation in the circled region and speech anxiety reduction, adjusting for baseline speech anxiety.

less activation in dorsomedial PFC (supplemental motor area) in the first half of extinction had greater reduction in public speaking anxiety.

Whole brain analyses revealed that individuals with greater reduction in public speaking anxiety also demonstrated less activation in left OFC, left fusiform gyrus, and left putamen to CS+ relative to CS- during the first half of extinction (Table 9). During the second half of extinction there were no regions where activation was associated with public speaking anxiety reduction.

Follow-up analyses to examine change in overall social anxiety (LSAS) similarly found that individuals with greater activation in the ventral ACC and less

Table 9. Extinction-Related Activations Associated with Public Speaking Anxiety Reduction from Baseline to Two Weeks Post-Exposure

Brain Region	**BA**	**Vol**	**X**	**Y**	**Z**	**Peak t**
Early Extinction						
R paracentral lobule / precentral gyrus / postcentral gyrus / middle cingulate	4; 31; 5	4416	14	-37	60	-3.98
L lingual gyrus / cuneus	17	2240	-30	-81	0	-3.56
L putamen		1344	-26	-13	20	-3.68
L fusiform gyrus	20	960	-42	-41	-8	-3.65
L cerebellum / parahippocampal Gyrus	36	896	-30	-29	-20	-3.97
L middle cingulate	31	832	-18	-29	40	-3.38
L OFC	10	768	-14	59	4	-3.90
Dorsomedial PFC*	6	704	6	-13	56	-3.80
Ventral ACC*	24; 32	384	-2	39	0	5.11
Medial OFC*	10	384	-14	63	0	-4.25
Late Extinction						
None						

Note. *indicates small volume corrected. Coordinates are in Talairach space. BA = Brodmann area, Vol = volume, R = right, L = left, PFC = prefrontal cortex, OFC = orbitofrontal cortex, ACC = anterior cingulate cortex

activation in dorsomedial PFC (supplemental motor area) in the first half of extinction had greater reduction in social anxiety from baseline to follow-up. Less activation in the left amygdala during early extinction was also associated with greater reduction in social anxiety following public speaking exposure. Accordingly, there was a moderate, though not statistically significant, trend towards individuals with greater ventral ACC activation also showing less amygdala activation ($r=-.37$, $p=.07$). In addition, whole brain analysis revealed that individuals with greater reduction in social anxiety demonstrated less activation in a wide range of regions including bilateral OFC, bilateral dorsolateral PFC, periaqueductal grey, and left anterior insula during early extinction (Table 10, Figure 5). During late extinction, only right anterior insula and medial OFC predicted reduction in social anxiety following exposure, with less activation associated with greater anxiety reduction (Table 10, Figure 5).

Results for both the public speaking and general social anxiety analyses were not substantially changed when depression symptoms (PHQ9) were included as a covariate. The general social anxiety (LSAS) analyses were also run without the three public speaking anxiety items, to examine whether results were driven by these items. Indeed, several regions listed in Table 10 were no longer associated with general social anxiety reduction when the three public speaking items were removed, including ventral ACC, medial OFC, left amygdala, and periaqueductal grey, although sub-threshold activation was evidence in ventral ACC. In addition, additional regions were uncovered where less activation during the second half of extinction was associated with greater reduction in non-public speaking social anxiety, namely

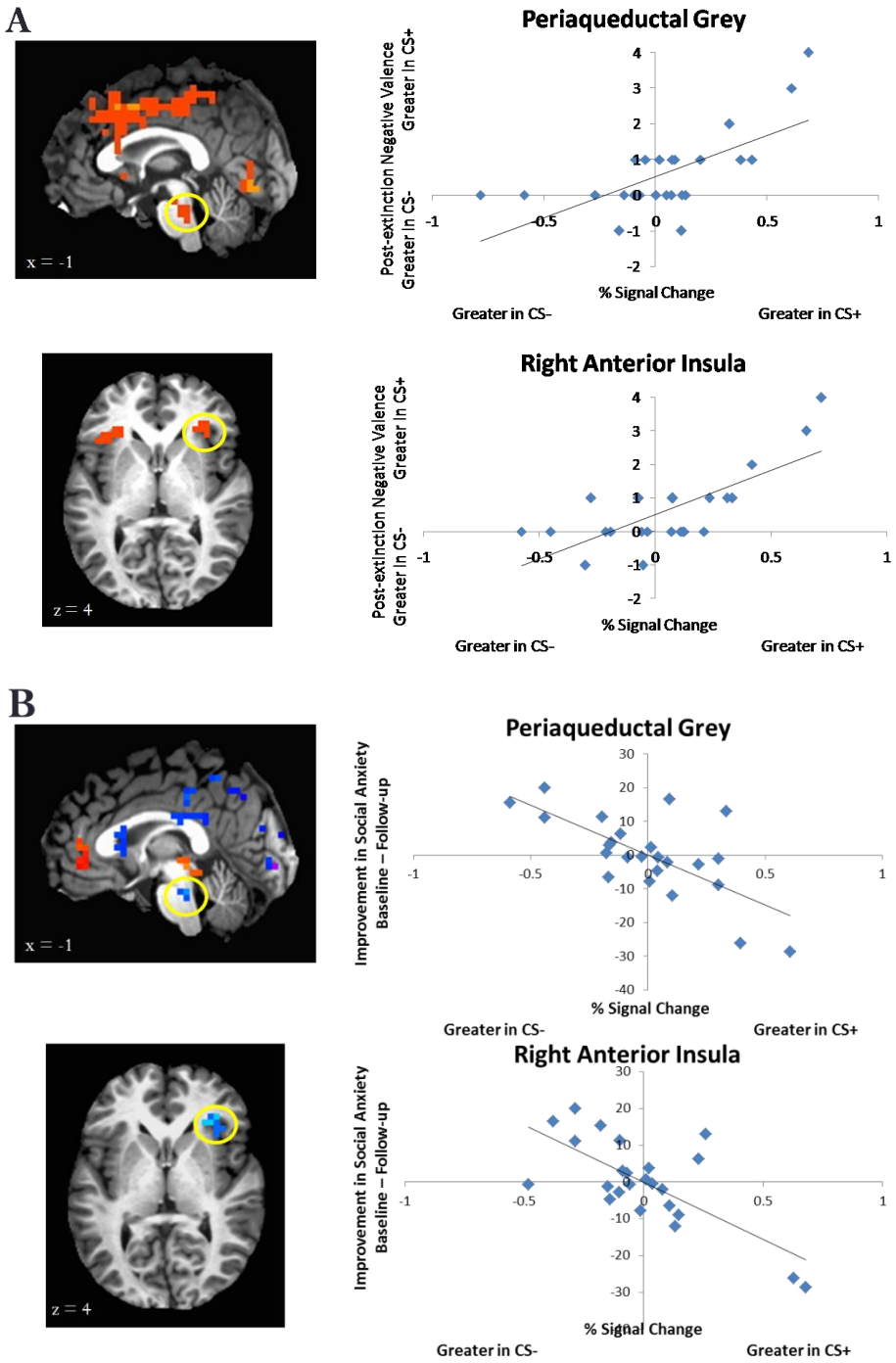

Figure 5. Periaqueductal grey and anterior insula activation associated with (A) post-extinction negative valence ratings and (B) social anxiety reduction from baseline to follow-up. Red color indicates positive association, blue indicates inverse association. Graphs indicate relationship between average activation in the circled region and (A) differential valence ratings or (B) anxiety reduction (adjusted for baseline social anxiety). Relationship with insula in (B) not significant after outlier removal.

Table 10. Extinction-Related Activations Associated with Social Anxiety Reduction from Baseline to Two Weeks Post-Exposure

Brain Region	**BA**	**Vol**	**X**	**Y**	**Z**	**Peak t**
Early Extinction						
Middle cingulate / paracentral lobule / medial precuneus	31; 23; 5	9600	-14	-25	48	-3.60
R dlPFC	9; 10	5056	14	39	28	-3.33
L putamen / anterior insula / white matter		4416	-22	11	16	-4.20
L OFC	10	4160	-22	39	12	-3.64
Bilateral caudate		3392	6	15	12	-3.79
Medial precuneus	7	2944	10	-49	40	-3.30
L cerebellum		2816	-18	-57	-20	-3.35
L lingual gyrus / cuneus	17; 18	2816	-14	-85	4	-3.60
White matter		2816	-34	-41	8	3.88
R OFC	10	2688	14	55	8	-2.99
L middle temporal gyrus†	21; 38	2368	-34	-5	-16	-3.49
R IFG / insula†	47	2368	42	-1	16	-3.28
R parahippocampal gyrus	36	2304	30	-49	0	3.30
L cerebellum		2176	-30	-65	-8	-3.50
R inferior parietal / supramarginal gyrus	40	1920	42	-49	32	-4.63
L inferior occipital gyrus	18	1856	-22	-81	8	-3.12
L posterior insula	13	1792	-30	-17	12	-3.26
L dlPFC	9	1792	-18	31	24	-3.09
Middle cingulate	24	1792	14	7	28	-3.56
R superior temporal gyrus	39; 22	1472	38	-45	24	-3.80
L precentral gyrus	4	1472	-38	-17	44	-2.89
L supramarginal gyrus	40	1408	-54	-53	28	-3.70
R superior temporal gyrus	21	1344	50	-17	-4	-3.16
R anterior temporal pole	38	1280	42	11	-20	-2.92
Dorsal PFC	6; 8	1280	10	15	56	-3.72
L cerebellum		1152	-26	-41	-24	-3.90
R cerebellum		1088	22	-53	-32	-5.80
Periaqueductal grey†		1088	-6	-29	-20	-4.66
R precentral / postcentral gyrus†	4; 3	1088	22	-21	56	-2.94
L superior temporal gyrus	38; 22	1024	-46	11	-8	-4.12
R putamen		1024	22	-9	16	-3.84
L middle temporal gyrus	22	1024	-50	-45	4	-4.82
R dlPFC	9	960	34	19	36	-3.45
R middle occipital gyrus / cerebellum	19	832	46	-65	-4	-3.21
Ventral/rostral ACC†	24; 32	832	-2	39	8	4.02
L lingual gyrus	19	832	-22	-61	12	-3.36

Table 10. Extinction-Related Activations Associated with Social Anxiety Reduction from Baseline to Two Weeks Post-Exposure, Continued

Brain Region	BA	Vol	X	Y	Z	Peak t
R inferior parietal lobule	40	832	50	-29	24	-3.03
R IFG	47	768	26	15	-8	-2.99
Superior colliculus		768	-2	-29	-4	4.11
Medial cuneus	18	768	10	-81	24	3.95
R cuneus	19	768	14	-81	36	-3.14
Medial PFC*	6	384	-2	-9	48	-3.15
L amygdala*†		256	-22	-5	-16	-3.39
Late Extinction						
R anterior insula	13	960	26	27	4	-3.30

Note. *indicates small volume corrected. †indicates region no longer significant when public speaking specific items removed. Coordinates are in Talairach space. BA = Brodmann area, Vol = volume, R = right, L = left, PFC = prefrontal cortex, OFC = orbitofrontal cortex, ACC = anterior cingulate cortex, IFG = inferior frontal gyrus, dlPFC = dorsolateral frontal cortex

anterior ventromedial PFC (x=6, y=59, z=-12) and dorsomedial PFC (x=-6, y=51, z=24).

Other Predictors of Anxiety Reduction

Exploratory correlation and linear regression analyses were conducted to examine whether longer term anxiety reduction was predicted by baseline self-report measures, valence ratings during the fear conditioning task, and/or anxiety ratings during exposure.

Baseline self-report. Baseline anxiety (PRCS, OASIS, LSAS), depression (PHQ-9), and disability (SDS) were all uncorrelated with change in public speaking anxiety (PRCS) from baseline to two-week follow-up ($p>.7$). These baseline self-report measures were also uncorrelated with change in overall social anxiety (LSAS), although baseline social anxiety demonstrated a moderate non-significant relationship with social anxiety change such that individuals with greater social anxiety had greater

anxiety reduction (r=.39, p=.06). This pattern of results was unchanged by excluding the three public speaking items from the LSAS (i.e, all non-significant except baseline modified LSAS, which was correlated with modified LSAS change r=.46, p=.02).

Demographic and clinical variables. Reduction in public speaking anxiety from baseline to follow-up was uncorrelated with age and did not differ between men and women. Reduction in social anxiety was similarly uncorrelated with age, though men reported greater social anxiety reduction than women following the exposure intervention (t(22)=2.23, p=.04, uncorrected). Average activation in the ventral ACC, PAG, or R anterior insula regions predictive of anxiety reduction (Figures 4 and 5) were unrelated to age or gender (p>.05).

Although baseline clinical measures differed based on social anxiety diagnostic status (generalized, non-generalized, or no diagnosis) as described above, neither reduction in anxiety from first to last speech nor reduction in public speaking or social anxiety from baseline to follow-up differed by social anxiety diagnostic status (p>.05). In addition, average activation in the ventral ACC, PAG, or R anterior insula regions predictive of anxiety reduction (Figures 4 and 5) did not differ based on social anxiety diagnostic status (p>.05).

Fear conditioning task negative valence ratings. Linear regressions were conducted with change in public speaking anxiety as the dependent variable and the difference in valence ratings between CS+ and CS- as the independent variable. Baseline public speaking anxiety was also included as an independent variable to control for initial severity. Both the overall regression and the independent

contribution of task ratings were non-significant for both acquisition and extinction. The same analysis was conducted with valence ratings predicting social anxiety change, controlling for baseline social anxiety. The overall regression for ratings collected post-acquisition neared significance ($F(2,21)=3.00$, $p=.07$, $R^2=.22$), however this was driven by a significant relationship between baseline anxiety and anxiety reduction ($t(21)=2.35$, $p=.03$), with no relationship between stimulus ratings and anxiety reduction ($p>.1$). The overall regression for ratings collected post-extinction was significant ($F(2,21)=7.64$, $p=.003$, $R^2=.42$), with both greater baseline anxiety ($t(21)=2.71$, $p=.01$) and a smaller difference between CS+ and CS- ratings post-extinction ($t(21)=-3.15$, $p=.005$) contributing to greater reduction of social anxiety. The pattern of results from these analyses was unchanged by removing the three public speaking items from the social anxiety total score.

Anxiety ratings during exposure. Peak anxiety ratings (SUDS) during the first speech was uncorrelated with change in peak anxiety from the first to the last speech ($p>.07$), and uncorrelated with change in public speaking anxiety and general social anxiety from baseline to two-week follow-up ($p>.2$). However, greater reduction in anxiety (SUDS) from the first to the last speech did predict greater reduction in public speaking anxiety from baseline to two-week follow-up ($r=.47$, $p=.02$). Change in anxiety ratings from the first to the last speech was uncorrelated with change in general social anxiety ($p>.3$).

Discussion

The present study examined the relationship between (1) public speaking anxiety and the neural bases of fear extinction learning, and (2) the relationship between neural bases of extinction learning and exposure success. The primary finding is that those individuals who, by self-report and neural activation, demonstrated better extinction learning also reported greater anxiety reduction following an exposure intervention. To our knowledge, this is the first time that the theoretical link between extinction learning and exposure efficacy has been demonstrated empirically.

Supporting this overall finding is the expected task effect on self-report ratings of the stimuli, and their relationship to brain activation during extinction. Fear acquisition and extinction effects were shown in valence and arousal ratings to CS+ relative to CS-, confirming the efficacy of the fear conditioning procedure. Furthermore, task ratings were associated with brain activations in expected fear-relevant structures including the amygdala, anterior insula, dorsal ACC, and PAG such that greater negative valence to CS+ relative to CS- was associated with greater activation in these regions. The massed exposure procedure also demonstrated significant anxiety reduction from baseline to two-week follow-up with a medium to large effect size; however, sufficient variability in this efficacy was present allowing for an examination of individual differences in anxiety reduction.

Hypothesis One: Anxiety Severity

The first hypothesis was that public speaking anxiety severity would be associated with greater amygdala and less vmPFC activation during fear extinction.

This hypothesis was not supported by the present data, in contrast to prior research (Pejic et al., 2013; Sehlmeyer et al., 2011). One key difference may be the use of non-social stimuli as CSs in the present study. Both prior investigations used neutral faces, which are known to yield greater amygdala activation in individuals with greater social anxiety (Cooney et al., 2006); this may have confounded the relationship with anxiety severity.

An unexpected finding that emerged from a follow-up, exploratory analysis of overall anxiety severity was that greater baseline severity was associated with greater activation in dorsomedial PFC during fear extinction. Although this finding was not hypothesized, past animal research has implicated more dorsal aspects of PFC in fear conditioning processes. Specifically, lesions to the dorsal aspect of the medial PFC have resulted in greater fear expression during both acquisition and extinction, with lesions to the ventral aspect affecting extinction learning only (Morgan & LeDoux, 1995). Therefore dorsomedial PFC may underlie a more generalized inhibition of fear responding that complements the more extinction-specific function of ventromedial PFC. Dorsomedial PFC activation may therefore represent a compensatory strategy during fear extinction in individuals with higher anxiety.

Hypothesis Two: Short-Term Exposure Outcomes

The second hypothesis was that less amygdala activation during extinction would predict greater short-term anxiety reduction (i.e., between-exposure habituation), however no relationship was found. The lack of a relationship between neural activation during fear extinction and between-exposure habituation is consistent

with theoretical and empirical work (e.g., Foa & Kozak, 1986; Craske et al., 2008) that suggests that the learning that occurs during exposure therapy contributes more to long-term anxiety reduction than does anxiety reduction during the exposure itself.

Hypothesis Three: Longer-Term Exposure Outcomes

The third hypothesis was that greater vmPFC activation during fear extinction would predict greater reduction of anxiety from baseline to two weeks following the exposure sessions. This hypothesis was supported by the present data, with greater activation in ventral ACC predicting reduction in both public speaking anxiety and social anxiety. This finding indicates that neural markers of better extinction learning can predict better exposure outcomes, consistent with theoretical accounts that fear extinction learning is the mechanism of exposure therapy (Craske et al., 2008). Although activation in this vmPFC region was associated with anxiety reduction following exposure, it was not associated with post-extinction self-report ratings of the stimuli. This suggests that activation in this region may underlie an unconscious learning process not captured by stimulus ratings that is important for exposure outcomes.

Exploratory analyses across the whole brain demonstrated that less PAG and anterior insula activation during extinction also predicted greater reduction in social anxiety. This is consistent with the notion that better extinction learning, in this case indexed by decreased activation in emotion related regions during extinction, predicts better exposure outcomes. In addition, individuals who demonstrated greater PAG and anterior insula activation during extinction also reported greater negative valence to

the CS+ even after extinction, lending further support to the idea that these regions' role in indexing extinction learning is what is driving the effect.

Finally, the exploratory analysis also uncovered greater parahippocampal gyrus activation during extinction as a predictor of greater reduction in social anxiety. This is consistent with the role of medial temporal structures in memory formation (R. Phillips & LeDoux, 1992), and further supports the pattern of better extinction learning associated with greater anxiety reduction following exposure.

Potential Alternative Explanations

The above results cannot be explained by anxiety severity at baseline, as all analyses of anxiety reduction controlled for baseline anxiety levels. The results can also not be accounted for by depression severity; adding a depression covariate did not alter key results. Anxiety reduction was uncorrelated with age and did not differ by baseline social anxiety diagnostic status.

Although analysis of the acquisition phase of the task is outside the scope of this project, several of the present results suggest that the effects are extinction specific rather than driven by acquisition effects. First, self-report ratings of the CS+ collected post-extinction were predictive of exposure outcomes, however post-acquisition ratings had no relationship to outcomes. Second, change in anxiety across the speech exposure predicted outcomes, however anxiety level to the first speech did not. Taken together, these results suggest two conceptually distinct clusters: (1) an anxiety severity and fear acquisition cluster, consisting of baseline anxiety severity,

anxiety to the first speech, and CS+ ratings following acquisition, and (2) an anxiety reduction and fear extinction cluster, consisting of anxiety reduction across the speech exposures, anxiety reduction from baseline to two-week follow-up, CS+ ratings following extinction, and brain activation during fear extinction.

Aim three examined not only reductions in public speaking anxiety but also general social anxiety, with the goal of uncovering potential predictors of generalization of fear reduction following the public speaking exposure. However, because the general social anxiety measure (LSAS) contains some public speaking related items, this analysis was also conducted with these items removed. Indeed, findings were changed by the removal of the public speaking related items, suggesting that these may have accounted for some of the effect. Specifically, activation in the ventral ACC was associated with reduction in public speaking anxiety, but was not associated with reductions in general social anxiety when the public speaking items were removed. However, an additional more anterior vmPFC region emerged as predictive of general social anxiety. This suggests that different sub-regions within vmPFC may be associated with fear extinction learning (i.e., ventral ACC) and generalization (i.e., anterior vmPFC).

Clinical Applications and Future Directions

Overall, the present results bridge the gap between extinction learning within laboratory and more ecologically valid contexts. Furthermore, the results provide an important step towards the mechanistic understanding of exposure therapy, a gold-standard treatment for anxiety disorders (Deacon & Abramowitz, 2004). Specifically,

the findings indicate that individuals whose brain activation dynamically adjusts to the presence or absence of aversive consequences may benefit most from brief exposure therapy. The individual differences approach to the present investigation contributes to the important goal of treatment matching (Eifert, Evans, & McKendrick, 1990). Accordingly, future research should examine whether fear extinction can reliably predict clinical outcomes and be used as a single subject prognostic test to guide treatment decisions (Ball, Stein, & Paulus, 2014).

Limitations

The present study examined a single high anxiety group only, with no healthy comparison subjects. Thus, conclusions about abnormalities in brain activation relative to non-anxious adults cannot be drawn. In addition, the lack of a normative anxiety group may have resulted in a restricted range in anxiety severity and therefore limited ability to detect an effect of severity on brain activation. The relatively small sample size also resulted in limited power to detect effects.

Secondly, no diagnosis was required for inclusion into the study and therefore some but not all participants met diagnostic criteria for social anxiety disorder. In addition, participants with greater than moderately severe depression were excluded. Future studies should include treatment seeking populations with common comorbidities (e.g., major depression) for greater generalizability of these results.

Thirdly, a single open-label treatment procedure was provided. Thus, whether brain activation during fear extinction predicts overall treatment responsiveness rather

than exposure efficacy in particular cannot be ruled out. Future studies should examine fear extinction learning as a moderator of outcomes in a randomized controlled trial.

Finally, the fear conditioning task was limited by the lack of an extinction recall condition, as well as by the use of self-report rather than physiological indicators of fear conditioning. Prior studies of fear extinction have used performance on a separate day extinction recall procedure as a key metric of extinction learning rather than relying only on data from the extinction procedure itself (e.g., Milad et al., 2009; Milad et al., 2007). In addition, a majority of prior fear extinction studies in anxiety disorders have utilized physiological indicators of fear responding, such as fear-potentiated startle or skin-conductance response (Lissek et al., 2005). Although the use of self-report as a dependent measure of fear conditioning is not unprecedented (Sehlmeyer et al., 2011), the present findings should be confirmed in future studies using more traditional physiological metrics.

Summary and Conclusions

In summary, high anxiety individuals who demonstrated better extinction learning based on both self-report and neural activation patterns also showed greater anxiety reduction following an exposure intervention. The theoretical link between extinction learning and exposure efficacy has never been demonstrated and provides an important step toward the mechanistic understanding of this intervention. Notably, neural activation was associated with anxiety reduction over a two-week follow-up period, but not anxiety reduction during the course of the exposure session, suggesting that the learning process that happens during exposure is more important than the

anxiety reduction during the exposure itself. Overall, the present results indicate that individuals who are most readily able to re-evaluate threat associations are those who may benefit most from brief exposure therapy. Future work should examine whether fear extinction can reliably predict clinical outcomes and be used as a single subject prognostic test to guide treatment decisions.

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