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Prefrontal and Amygdala Engagement during Emotional Reactivity and Regulation in Generalized Anxiety Disorder

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Abstract

Background: Emotion dysregulation is prominent in generalized anxiety disorder (GAD), characterized clinically by exaggerated reactivity to negative stimuli and difficulty in down-regulating this response. Although limited research implicates frontolimbic disturbances in GAD, whether neural aberrations occur during emotional reactivity, regulation, or both is not well understood.

Methods: During functional magnetic resonance imaging (fMRI), 30 individuals with GAD and 30 healthy controls (HC) completed a well-validated explicit emotion regulation task designed to measure emotional reactivity and regulation of reactivity. During the task, participants viewed negative images ('Look-Negative' condition) and, on some trials, used a cognitive strategy to reduce negative affective response ('Reappraise' condition).

Results: Results from an Analysis of Variance corrected for whole brain multiple comparisons showed a significant group x condition interaction in the left amygdala and left inferior frontal gyrus (IFG). Results from post-hoc analyses showed that the GAD group engaged these regions to a greater extent than HCs during Look-Negative but not Reappraise. Behaviorally, the GAD group reported feeling more negative than the HC group in each condition, although both groups reported reduced negative affect following regulation.

Limitations: As comorbidity was permitted, the presence of concurrent disorders, like other anxiety disorders and depression, detracts our ability to classify neural engagement particular to GAD alone.

Conclusions: Individuals with GAD exhibited over-engagement of amygdala and frontal regions during the viewing of negative images, compared to HCs. Together, these aberrations may indicate that deficits in emotional reactivity rather than regulation contribute to emotion dysregulation in those with GAD.

Keywords

generalized anxiety disorder; affect; emotion regulation; reappraisal; fMRI

Introduction

Generalized anxiety disorder (GAD) is a prevalent, disabling and difficult disorder to treat (Gould et al., 2004; Hofmann and Smits, 2008; Mennin et al., 2005). While Cognitive Behavioral Therapy (CBT) is an empirically-supported psychotherapy for this disorder, symptoms persist in upwards of 50% of patients who complete treatment (Fisher, 2006; Hunot et al., 2007; James et al., 2013). Metacognitive therapy, which attempts to reduce 'reactivity' to salient cues and increase emotion regulation skills, fares better (Heiden, 2013), but still leaves upwards of 23% of those with GAD with residual symptoms (van der Heiden et al., 2012). In addition, although various pharmacotherapies have been used to treat the disorder, up to 50% of patients with GAD do not experience a significant symptom decline following treatment (Buoli et al., 2013). Together these data demonstrate that a sizable portion of the GAD population remains symptomatic, despite undergoing currently available interventions.

One possible explanation for mixed efficacy in the treatment of GAD is that the neural underpinnings of symptoms central to the disorder remain largely unknown and, therefore, possibly untreated. Notably, while excessive and uncontrollable worry and an array of somatic symptoms (e.g., restlessness, muscle tension) characterize GAD (American Psychiatric Association, 2013), these complaints have been hypothesized to stem from emotion dysregulation as a common feature (Mennin et al., 2015, 2007, 2005). Even so, not much is known about the neurobiology associated with emotion dysregulation in GAD. Further, in defining what is meant by emotion dysregulation, this symptom consists of two separate, yet related, abnormalities: atypical emotional reactivity and regulation of reactivity (Lewis et al., 2010). Thus, emotional dysregulation observed in GAD may be the result of neural perturbations tied to exaggerated reactivity to salient stimuli and/or deficit or inefficient capacity to dampen emotional reactivity. As a result, while emotion dysregulation may be a central component of GAD, more information is needed to qualify the extent to which exaggerated reactivity and deficiency in down-regulation characterize emotion dysregulation as it presents in this disorder.

Evidence from clinical and behavioral studies help qualify the nature of emotional dysregulation as it appears in GAD. This work documents that GAD is associated with intense, inappropriate (i.e., out of context) affective reactivity (Mennin et al., 2007, 2005). Perhaps as a result, compared to healthy controls, those with GAD report feeling more negative when exposed to emotional stimuli (e.g., negative faces) (Kirschner et al., 2016; Mennin et al., 2005; Turk et al., 2005) and experience more physiological symptoms of anxiety during and following negative mood inductions (e.g., upset stomach, feeling restless) (MacDonald et al., 2015). Exaggerated negative reactivity may also be in part explained by increased attention towards emotional stimuli, as studies have demonstrated attentional bias to negative images (Roy et al., 2008; Weinberg and Hajcak, 2011) and emotional expressions

in this population (Bui et al., 2017). In addition to exaggerated reactivity to negative stimuli, however, individuals with GAD also self-report difficulty in down-regulating negative affect when intending to do so (Amstadter, 2008; Cisler et al., 2010; Mennin et al., 2009, 2005; Salters-Pedneault et al., 2006; Turk et al., 2005), suggesting that deficits related to explicit regulation of negative affect are also present. It has also been shown that self-reported deficiency in emotion regulation uniquely predicts GAD symptoms when controlling for underlying symptoms of affect reactivity (Mennin et al., 2005), suggesting that regulation-related deficits may be a unique element of emotion dysregulation in GAD. Nevertheless, as theoretical work suggests that deficits in regulating emotions in GAD may stem from underlying exaggerated affective reactivity (Mennin et al., 2007), more work is needed to help clarify the prominence of each component of emotion dysregulation in GAD. Neuroimaging work that measures the extent to which neural functioning is compromised during reactivity to and regulation of negative content may help clarify and extend this work.

The neural mechanisms of reactivity to negative stimuli are well-characterized in healthy individuals. This work demonstrates a central role for the amygdala, a region known for its involvement in the detection, encoding and organization of response to salient stimuli (Anderson et al., 2003; Cunningham and Brosch, 2012; Phelps and LeDoux, 2005; Wilson-Mendenhall et al., 2013). The amygdala is activated during negative picture viewing (Hariri et al., 2002; Ochsner et al., 2009) and activation in the region correlates with self-reported negative affect (Ochsner et al., 2009) and peripheral indices of emotional arousal, like skin conductance (Hariri et al., 2002; Raine et al., 1991; Williams et al., 2004, 2001). Therefore, amygdala engagement is related to both objective and subjective markers of an emotional experience.

In line with observations that anxiety disorders are qualified by greater emotional reactivity (Barlow, 2004), there is substantial neuroimaging evidence that, compared to healthy controls, common, disabling affective disorders such as social anxiety disorder (SAD) (Carré et al., 2014; Fonzo et al., 2015; Stein et al., 2007) and panic disorder (PD) (Feldker et al., 2016; Fonzo et al., 2015; Rauch et al., 2003; van den Heuvel et al., 2005) are characterized by hyper-engagement of the amygdala during the viewing of negative images. However, despite exaggerated reactivity reported clinically in GAD, results are inconsistent with regard to whether this disorder is also characterized by exaggerated amygdala response. While some research has found hyper-engagement of the amygdala during viewing of negative images in GAD compared to healthy controls (HCs) (Fonzo et al., 2015; McClure et al., 2007; Monk et al., 2008; Nitschke et al., 2009), there are also cases of hypo-engagement (Andreescu et al., 2011; Blair et al., 2008) or a failure to find group differences (Whalen et al., 2008). Despite the fact that excessive reactivity to negative stimuli is a prominent symptom of GAD, given such contrasting findings, more work is needed to better understand the neurobiology of emotional reactivity as it presents in this disorder.

In healthy individuals, mechanisms of explicit emotion regulation are frequently studied using the strategy of cognitive reappraisal, in which individuals modulate the emotional salience of a stimulus by changing its meaning (Gross, 2013; Ochsner et al., 2012). Cognitive reappraisal is considered an adaptive form of emotion regulation, based on research demonstrating its positive association with psychological health and well-being

(Cutuli, 2014). As a result, the neural mechanisms that underlie cognitive reappraisal are well-known in individuals without psychopathology. Studies have consistently shown that cognitive reappraisal involves recruitment of several cortical regions, including the inferior frontal gyrus (IFG), dorsolateral PFC (DLPFC), dorsomedial PFC (DMPFC), ventrolateral PFC (VLPFC), anterior cingulate cortex (ACC), middle and superior temporal gyri, and parietal regions including the angular gyrus (Buhle et al., 2014; Kohn et al., 2014; Messina et al., 2015; Ochsner et al., 2012). The recruitment of specific frontal regions during reappraisal (e.g., DLPFC, VLPFC, ACC) is also associated with reduced amygdala responding, thought to reflect down-regulation of the negative emotional experience (Banks et al., 2007; Eippert et al., 2007; Frank et al., 2014; Ochsner et al., 2012). Though the inverse relationship between cortical engagement and amygdala activity is consistent with a “top-down” model of emotion regulation (Banks et al., 2007; Eippert et al., 2007; Frank et al., 2014; Ochsner et al., 2012), there are also reports of amygdala engagement during reappraisal in non-psychiatrically-ill samples (McRae et al., 2012; Nelson et al., 2015). Inconsistent findings may reflect sub-processes that underlie regulation, one of which involves establishing and elaborating on the emotional meaning(s) of a stimulus so that it can be subsequently re-interpreted (McRae et al., 2012). Therefore, “success” in emotion regulation at the neural level may not be best qualified by changes in amygdala responding (Morawetz et al., 2016) as regulation may be associated with amygdala response or attenuated activation.

Relative to other internalizing disorders such as SAD and PD (Ball et al., 2013; Blair et al., 2012; Goldin et al., 2008; Reinecke et al., 2015; Zilverstand et al., 2016; Ziv et al., 2013), less is known in GAD regarding neural activity during cognitive reappraisal. In fact, only two fMRI studies of explicit emotion regulation in GAD have been conducted and findings from this work must be considered in light of methodological complications that make it difficult to draw firm conclusions about the nature of aberrations in GAD. For instance, one study found that those with GAD exhibited less activation in frontal (e.g., DLPFC, DMPFC) and visual areas (e.g., middle occipital gyrus, fusiform gyrus) compared to HCs when using reappraisal to decrease negative reactivity to aversive images (Ball et al., 2013). However, in this study, the HC group did not show *differential* engagement of these regions between the viewing of negative images and when using reappraisal to down-regulate, making it difficult to conclude that under-engagement of these regions represented a deficiency related to reappraisal in GAD. In another study, HCs selectively engaged the dorsal ACC and inferior and superior parietal cortices to a greater extent than those with GAD when using reappraisal to down-regulate reactivity to negative images compared to negative reactivity as a baseline condition (Blair et al., 2012). However, the GAD group displayed elevated engagement in these regions across conditions of negative reactivity and reappraisal (Blair et al., 2012). As a result, aberrations in GAD may be explained by reduced capacity to effectively modulate limbic hypersensitivity to negative stimuli as an underlying disturbance, although this possibility was not fully explored by the authors (Blair et al., 2012). In both studies, while GAD participants reported feeling more negative compared to HCs across conditions of negative reactivity and regulation, negative affect was reduced when using reappraisal in both groups, suggesting an ability to regulate when instructed in GAD (Ball et al., 2013; Blair et al., 2012). Nevertheless, findings from this small body of

work suggest a foci for neural abnormalities in GAD when viewing negative images and when using reappraisal for emotion regulation. It is still unclear the extent to which amygdala hyper-reactivity occurs in GAD, and whether cortical aberrations during regulation of reactivity are specific to down-regulation, or whether atypical response during the viewing of negative images also occurs in these regions (Ball et al., 2013; Blair et al., 2012). That is, while prior work offers clues as to which brain regions may be impaired in GAD related to emotion dysregulation, more work is needed to decipher the extent to which neural aberrations occur during emotional reactivity and regulation of this reactivity.

The goal of the current study was to examine the neural correlates of emotional reactivity and cognitive reappraisal as a technique to achieve emotional regulation in GAD. Based on existing literature and theory, we hypothesized exaggerated amygdala reactivity in GAD, relative to HCs, when viewing negative images. During reappraisal, we hypothesized that individuals with GAD, compared to HCs, would exhibit less engagement in cortical regions implicated in emotion regulation (e.g., IFG, DLPFC, DMPFC). Behaviorally, we expected the GAD group to report feeling more negative than HCs in both the emotional reactivity and reappraisal conditions, reflecting greater subjective reactivity to negative stimuli and deficiency in dampening emotional response to negative stimuli. We also conducted post-hoc analysis to explore potential relationships between self-reported affect reactivity and the neural correlates of emotional reactivity and reappraisal within GAD and HC groups.

Methods and Materials

Participants

Participants were between 18 and 65 years of age and free of major medical or neurologic illness as confirmed by a Board Certified physician and were recruited by means of advertisement throughout the community. Interested participants completed a phone screen followed by a psychiatric evaluation during which time they reviewed the consent form as approved by the local Institutional Review Board at the University of Illinois at Chicago. After attaining consent, participants completed the self-report Penn-State Worry Questionnaire (PSWQ; Meyer et al., 1990) and met with a Master's-level clinician who administered the Structured Clinical Interview for the DSM-IV (SCID-IV; First et al., 2002), Hamilton Anxiety Rating Scale (HAM-A; (Hamilton, 1959) and Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960). Diagnosis of current GAD was based on the SCID-IV and HCs were required to not have current, or history of, an Axis I disorder as determined by the SCID. All participants were free of psychotropic medications and none were receiving concurrent psychotherapy. None of the participants tested positive for drugs. Exclusion criteria for all participants included cognitive dysfunction (traumatic brain injury, mental retardation, organic mental syndrome, dementia), history of major psychiatric illness (e.g., bipolar disorder, psychotic disorder, pervasive developmental disorder), current substance dependence (within 6 months of study), and contraindications to functional magnetic resonance imaging (fMRI) (e.g., pregnancy, claustrophobia, non-removable ferrous objects). Participants were compensated for their time and all procedures complied with the Helsinki Declaration.

Sixty-nine individuals (GAD: $N = 35$; HC: $N = 34$) participated in the study. One HC participant was excluded from further analyses due to excessive motion during fMRI (groups did not differ in movement realignment parameters) and seven (GAD: $n = 5$; HC: $n = 2$; 10% of original sample) due to missing or incomplete negative affect ratings that were collected online during the fMRI task to confirm task validity, resulting in a final sample size of 60 participants ($n = 30$ GAD, $n = 30$ HC). In the final GAD sample, comorbidities included social anxiety disorder ($n = 13$), major depressive disorder ($n = 6$), panic disorder ($n = 3$), specific phobia ($n = 3$), PTSD ($n = 2$), and obsessive-compulsive disorder ($n = 2$). All participants were required to be free from psychotropic medication for at least four weeks prior to testing. In assessing treatment history, nine patients reported participating in psychotherapy, three patients reported past-use of antidepressants, and one patient reported past use of benzodiazepines. See Table 1 for complete listing of sample demographics, displayed by group.

fMRI task

During fMRI scanning, participants completed a block-design Emotion Regulation Task (ERT), based on paradigms previously validated in other labs (Ochsner et al., 2002) and ours (Fitzgerald et al., 2016; MacNamara et al., 2016; Phan et al., 2005; Rabinak et al., 2014). During ERT, participants were shown 64 negative and 32 neutral images from the International Affective Picture System (IAPS; Lang et al., 2008) across three conditions (Reappraise, Look-Negative, Look-Neutral). Participants were instructed to: 1) use a cognitive strategy to reduce negative affect to aversive images ('Reappraise' condition); 2) attend to the emotional state elicited by aversive images ('Look-Negative' condition); or 3) view neutral images ('Look-Neutral' condition). Prior to scanning, participants were instructed on the strategy of reappraisal (Ochsner et al., 2002; Phan et al., 2005); all conditions were practiced with images not used in the experiment to confirm understanding of task instructions.

The task consisted of eight 20 s blocks of each condition (four images presented for 5 s each without inter-stimulus interval). Blocks were interspersed by 20 s blocks of a white fixation cross, shown on a black background to enable the hemodynamic response to return to baseline. Block order was pseudo-randomized over the course of two separate runs, with each run lasting a total of five minutes. Prior to each block, an instruction screen ("Reappraise" or "Look") was presented for 5 s. To assess behavioral responses, following each block, participants viewed a screen that asked them to answer the question "How negative do you feel?" for the collection of online subjective negative affect ratings. Participants indicated their response on a 5-item Likert scale (1 = not at all; 5 = extremely) via a 5-button response with their dominant hand.

Behavioral data analysis

To explore group, condition, and group x condition effects of subjective negative affect rating scores, we employed a 2 (group: HC, GAD) x 3 (condition: Reappraise, Look-Negative, Look-Neutral) mixed measures ANOVA with condition as a repeated measure. Post-hoc *t*-tests were conducted to examine the presence and direction of group differences in negative affective state at each condition.

fMRI acquisition and preprocessing

FMRI scanning was performed on a 3T GE 3.0 Tesla GE MR 750 scanner (General Electric Healthcare; Waukesha, WI) using a standard radiofrequency coil. Whole-brain functional images (i.e., blood oxygen level-dependent [BOLD]) were collected using the following parameters: TR = 2s, TE = 25 ms, flip angle = 90°, field of view = 22 × 22 cm², acquisition matrix 64 × 64; 44 axial, 3-mm-thick slices with no gap. The first 4 volumes from each run were discarded to allow magnetization to reach equilibrium.

Conventional preprocessing steps were completed in Statistical Parametric Mapping (SPM8) software package (Wellcome Trust Centre for Neuroimaging, London www.fil.ion.ucl.ac.uk/spm). Images were temporally corrected to account for slice time acquisition differences and spatially realigned to the mean image to correct for head movement while motion realignment parameters were entered as regressors of no-interest to control for minimal head movement during scanning. Functional images from all participants included in analysis met criteria for quality with minimal motion correction (movements were < 3 mm and < 3 degrees rotation in any one direction). Images were then normalized to a Montreal Neurological Institute (MNI) template using the echo-planar imaging template, resampled to 2 × 2 × 2 voxels and smoothed with an 8 mm isotropic Gaussian kernel.

fMRI data analysis

A general linear model was applied to the time series, convolved with the canonical hemodynamic response function and with a 128 s high-pass filter. Blocks of Reappraise, Look-Negative, and Look-Neutral were modeled separately in relation to implicit baseline (i.e., fixation cross), the effects of which were estimated for each voxel for each participant and taken to the second level for random effects analysis. Our primary objective was to dissociate emotional reactivity from emotion regulation (i.e., reappraisal); therefore, neural activity in the presence of negative images during Look-Negative and Reappraise were modeled separately, each contrasted with a non-emotional/neutral baseline. Specifically, contrasts of interest were Look-Negative (>Look-Neutral) and Reappraise (>Look-Neutral).

To examine main effects and interactions, we conducted 2 (Group: HC, GAD) × 2 (Condition: emotional reactivity, emotion regulation) mixed measures ANOVA with condition as a repeated measure. Following recent guidelines, created in response to concerns about false positives resulting from lenient significance thresholds (Eklund et al., 2016; Woo et al., 2014), clusters from the ANOVA were considered significant only when they surpassed correction of multiple comparisons across the entire brain (e.g., a whole-brain gray matter mask [volume = 1,459,304 mm³]) as determined via simulation using the 3dClustSim utility (10,000 iterations; updated and ‘bug-free’ on December 2015; [https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html]; Cox, 1996). Significance at $\alpha < 0.05$ and a voxel threshold of $p < 0.001$ yielded a minimum cluster size of 60 voxels (volume = 480 mm³) for the mixed measures ANOVA analysis. Clusters identified in significant interactions were followed up by comparing activation in these regions between groups for each condition separately.

To clarify the direction of effects, BOLD signal responses (parameter estimates, β -weights in arbitrary units [a.u.] of activation) were extracted using the SPM MarsBar toolbox (Brett et al., 2002) from functional clusters within *a priori* regions (e.g., IFG, DLPFC, DMPFC, VLPFC, ACC, temporal, and parietal gyri, and amygdala) that survived correction in the Group x Condition interaction. Parameter estimates were then imported into the Statistical Package for the Social Sciences (SPSS, Version 22) in order to perform simple effects analysis (e.g., independent samples *t*-tests). All analyses in SPSS were two tailed at alpha level 0.05.

To explore possible relationships between significant clusters of activation and behavioral ratings, self-reported emotion regulation ability was calculated by subtracting Reappraise from Look-Neutral, while self-reported emotional reactivity was calculated by subtracting Look-Negative from Look-Neutral. These values were submitted to two-tailed Pearson's correlations with parameter estimates extracted from ROIs described above.

Results

Affective results and manipulation check

We found main effects of Group ($F(1, 58) = 17.21, p < 0.001$) and Condition ($F(2, 116) = 138.11, p < 0.001$) for affect reports, which were moderated by a significant Group x Condition interaction ($F(2, 116) = 6.41, p < 0.01$). To explore the significant interaction, follow-up independent samples *t*-tests determined that, compared to HCs, individuals with GAD reported more negative affect following both Look-Negative ($t(58) = 3.37, p < 0.01$) and Reappraise ($t(58) = 4.18, p < 0.001$) conditions. In contrast, groups did not differ in subjective negative affect ratings following Look-Neutral ($t(58) = 1.24, p > 0.22$). Within group analyses indicated a reduction in negative affect following Reappraise compared to Look-Negative conditions in both HC ($t(29) = 3.62, p < .01$) and GAD groups ($t(29) = 2.39, p < .03$). Figure 1 displays mean subjective negative affect ratings following Look-Neutral, Look-Negative, and Reappraise trials, displayed by group.

fMRI results

Whole-brain voxel-wise ANOVA results revealed significant main effects of Group and Condition, which were moderated by a significant Group x Condition interaction in the left amygdala (peak MNI coordinate: $-26, -4, -20$; $Z = 3.93$; volume = $3,640 \text{ mm}^3$; $p < 0.05$ corrected) and left IFG (peak MNI coordinate: $-20, 34, -16$; $Z = 3.83$; volume = $1,808 \text{ mm}^3$; $p < 0.05$ corrected). We did not observe a significant interaction in other *a priori* regions. The main effect of Group revealed differential engagement in the middle and inferior frontal gyri as well as superior and middle temporal gyri while the main effect of Condition showed differential engagement in the inferior frontal gyrus and the superior and temporal gyri, considered *a priori* regions (see Table 2 for all results).

To aid in the interpretation of these interactions, post-hoc independent samples *t*-tests were conducted in SPSS for amygdala and IFG. Post-hoc results showed that the GAD group, compared to HCs, exhibited more activation in the left amygdala during emotional reactivity ($t(58) = 3.26, p < 0.01$), but not regulation ($p > 0.91$). Figure 2, Panels A and B display the

whole-brain corrected left amygdala cluster and mean parameter estimates of activation extracted from the left amygdala displayed by condition and group, respectively.

For the left IFG interaction, post-hoc results revealed that the GAD group, compared to HCs, exhibited more activation in this region during emotional reactivity ($t(58) = 2.41, p < 0.02$) but not regulation ($p > 0.42$). Figure 2, Panels A and B display the whole-brain corrected IFG cluster and mean parameter estimates of activation extracted from the left IFG displayed by condition and group, respectively.

To explore the relationship between left amygdala and left IFG activity, Pearson's correlations were performed in SPSS in order to better understand their joint involvement during emotional reactivity and reappraisal. Here it was found that, within each group, the left amygdala and left IFG were positively correlated during emotional reactivity (HC: $r(28) = 0.79, p < 0.001$; GAD: $r(28) = 0.39, p < 0.04$) and regulation (HC: $r(28) = 0.49, p < 0.01$; GAD: $r(28) = 0.47, p < 0.01$). To determine whether the strength of these correlations differed significantly between HC and GAD groups, correlation coefficients were converted into z-scores using Fisher r-to-z transformation and, factoring in sample sizes for each group, z-scores were compared. The strength of this relationship was not significantly different between HC and GAD groups during cognitive reappraisal ($p > 0.92$). However, the positive relationship was stronger for HCs during emotional reactivity ($Z = 2.42, p < 0.02$). Figure 3, Panels A and B displays the positive correlation between left amygdala and left IFG clusters for each group during conditions representing emotional reactivity and reappraisal, respectively

Correlation between fMRI results and self-reported negative affect

Self-reported affect reactivity was not related to beta estimates drawn from the left amygdala during Look-Negative (HC: $p > 0.32$; GAD: $p > 0.72$) or Reappraise (HC: $p > 0.89$; GAD: $p > 0.88$). Beta estimates drawn from the left IFG during Look-Negative (HC: $p > 0.17$; GAD: $p > 0.59$) and Reappraise (HC: $p > 0.15$; GAD: $p > 0.51$) were also not related to self-reported affect reactivity.

Similarly, self-reported regulation ability was not related to beta estimates drawn from the left amygdala during Look-Negative (HC: $p > 0.51$; GAD: $p > 0.45$) or Reappraise (HC: $p > 0.96$; GAD: $p > 0.90$). In addition, beta estimates drawn from the left IFG during Look-Negative (HC: $p > 0.87$; GAD: $p > 0.22$) and Reappraise (HC: $p > 0.64$; GAD: $p > 0.15$) were also not related to self-reported regulation ability,

Discussion

The present study tested whether individuals with versus without GAD differed in brain functioning and subjective negative affect during the viewing of negative images (i.e., emotional reactivity) and when using a cognitive approach to reduce negative affect evoked by negative images (i.e., emotion regulation). Behaviorally, both GAD and control participants reported reductions in subjective negative affect as a function of reappraisal, indicating the instruction to regulate emotion was followed. However, the GAD group, relative to healthy control (HC) group, reported feeling more negative during both the

emotion reactivity and regulation phase. As a complement to these behavioral results, whole-brain results showed individuals with GAD exhibited greater left amygdala and left IFG activation relative to HCs during emotion reactivity. Yet, during cognitive reappraisal, left amygdala and left IFG activity were similar between GAD and controls.

The finding that the GAD group reported feeling more negative during reactivity to negative stimuli is consistent with prior research (Ball et al., 2013; Blair et al., 2012; Decker et al., 2008; Mennin et al., 2009, 2005), as is the finding that they experience reduced negative affect following reappraisal (Aldao and Mennin, 2012; Ball et al., 2013; Blair et al., 2012). However, our evidence that the GAD group still reported feeling more negative following reappraisal compared to HCs suggests that negative affect remained at an elevated level.

At the neural level, GAD individuals engaged the left amygdala to a greater extent during the viewing of negative images, a result that is also consistent with prior research (Fonzo et al., 2015; McClure et al., 2007; Monk et al., 2008; Nitschke et al., 2009; although see Andreescu et al., 2011; Blair et al., 2008; Whalen et al., 2008) and suggests that individuals with GAD are more reactive to negative stimuli. That we did not find evidence for a relationship between self-reported affect and engagement of the amygdala is consistent with prior work (Banks et al., 2007), and suggests that these disturbances are not linearly related (or subjectively perceived) in this sample. In contrast, hyper-engagement of the left amygdala in those with GAD compared to HCs during reappraisal was not found. GAD also engaged the left IFG to a greater extent compared to HCs during the viewing of negative images, a finding that coincides with prior research that found increased engagement in GAD compared to controls of the lateral middle frontal gyrus during the viewing of angry faces (Blair et al., 2008). This finding is also consistent with prior research documenting a similar effect in individuals with SAD, suggesting that this deficit is not specific to GAD but may be evident in multiple anxiety disorders (see Etkin and Wager, 2007 for a review). In addition, increased connectivity between the amygdala and DLPFC at rest has been found in those with GAD, with stronger connectivity between these regions in patients with the least symptoms (Etkin et al., 2009). Therefore, increased amygdala-frontal connectivity may represent compensatory functioning in GAD, which may be occurring due to elevated affective reactivity. As less gray matter volume in the IFG has also been documented in adults (Shang et al., 2014) and adolescents with GAD (Strawn et al., 2015), functional abnormalities in this region may stem from aberrations in the structure these regions.

Prior work highlights that the left IFG plays a role in emotional reactivity by generating inner speech (Geva et al., 2011; Jones and Fernyhough, 2007; Morin and Hamper, 2012), which may help individual categorize emotions (Kohn et al., 2014). Over-engaging this region during the viewing of negative images may signify greater appraisal of such stimuli in GAD, but may also be related to difficulty in identifying and labeling content as negative, as the disorder is also associated with problems identifying primary emotions like anger and fear (Mennin et al., 2005; Turk et al., 2005). Nevertheless, we did not find a relationship between the left IFG and feelings of negative affect, suggesting that greater engagement of this region is not linearly related to subjective feelings of negative affect. More work is needed to directly test whether over-engagement of the left IFG during negative emotional

experience relates to greater appraisal of emotion or, alternatively, difficulty in perceiving and/or classifying emotional content.

Altogether, this work demonstrates that atypical exaggerated engagement of the left amygdala and the left IFG may signify that GAD individuals exhibit greater reactivity to negative stimuli than controls (Buhle et al., 2014; Kohn et al., 2014; Messina et al., 2015; Ochsner et al., 2012). This proposal is supported by the fact that engagement of the left IFG was positively related to engagement of the left amygdala across conditions and in both HC and GAD groups, suggesting that engagement of the left IFG signals hyper-involvement of sub-processes in the experiencing of negative affect, but not the direct inhibition of negative affect.

To note, we also found in the present study that HCs engaged the left amygdala to a greater extent during cognitive reappraisal compared to emotional reactivity, a finding that is in line with prior work in healthy participants (McRae et al., 2012; Nelson et al., 2015). Cognitive reappraisal involves many sub-processes, one of which involves establishing and elaborating on the emotional meaning(s) of a stimulus so that it can be subsequently re-interpreted (McRae et al., 2012). The amygdala is likely involved in increasing salience and/or emotional meaning that is a necessary precursor to reappraisal (McRae et al., 2012). However, our inability to separate initial appraisal and subsequent reappraisal processes in the block-design version of our task makes it difficult to draw firm conclusions regarding the nature of amygdala's precise involvement in the reappraisal condition.

Clinical and behavioral work demonstrates that emotion regulation disturbances in GAD involve excessive reactivity to negative stimuli and a failure to effectively downregulate negative affective state (Mennin et al., 2005). The current findings expand on this work by demonstrating the importance of underlying neural correlates of this exaggerated negative reactivity in GAD. In addition, we observed GAD did not differ from HCs at the neural level during reappraisal, which suggests GAD is not necessarily associated with disturbances when using this strategy to reduce negative affective state. Both results have important implications for treatment. For instance, Emotion Regulation Therapy (ERT) is a recently developed psychosocial intervention for patients with GAD (Mennin et al., 2015), which attempts to reduce inappropriate/excessive emotional reactivity by teaching patients to increase motivational awareness, develop a range of emotion regulatory approaches, and generate new learning strategies (e.g., contextual learning consequences) via exposure to novel situations (Mennin et al., 2015). Although more work is needed to fully study ERT's efficacy, an initial open trial of ERT for individuals with GAD ($n=21$) has yielded promising preliminary results (Mennin et al., 2015). Taking our results into consideration, ERT mechanisms of action may involve modulating affective reactivity and provide multiple pathways (both direct and indirect targets) to improve emotion regulation by targeting aberrant regulation beyond reappraisal. In other words, patients engage in various forms of maladaptive regulation strategies (e.g., rumination, suppression, avoidance) (Aldao and Nolen-Hoeksema, 2013), which may be benefitted by the range of regulatory approaches taught in ERT. Certainly, much work is needed to understand how such treatment interventions can target specific neural deficits.

Results of the present study should be viewed in light of several limitations. First, several comorbidities were included in the present GAD sample, detracting our ability to classify neural engagement particular to GAD alone. Nonetheless, given the high comorbidity rate for GAD (Noyes, 2001), these findings are generalizable to those with GAD in community and clinical settings. Second, successful regulation using reappraisal was measured with self-report of negative affect, which without other corroborative evidence can be limited in its ability to accurately measure physiological affective response (Levenson, 2006). Third, images were of general negative content; therefore, results may not generalize to ideographic material or to positive stimuli.

Overall, findings support the proposal that emotion dysregulation in GAD is associated with aberrant amygdala and prefrontal cortical engagement (Mochcovitch et al., 2014; Zilverstand et al., 2016), qualified further by noting that over-engagement of these regions reflects deficits in reactivity to these images rather than the explicit regulation of these responses. A positive relationship between left amygdala and left IFG engagement in the present study suggests that hyper-engagement of these regions in GAD is related to concurrent heightened processing of and interpretation of negative stimuli. Based on this evidence and low response rate to standard psychotherapies for the treatment of GAD (e.g., CBT), interventions that specifically target exaggerated reactivity to negative stimuli and provide a range of approaches directed toward modifying emotion dysregulation may be more effective at reducing GAD symptoms and improving functioning in this population (Mennin et al., 2015).

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Figure 1.

Mean subjective negative affect ratings following each condition (Look-Neutral, Look-Negative, Reappraise), organized by group. Greater numbers on the y-axis indicate greater negative affect ratings. Error bars reflect ± 1 standard error. HC = healthy control; GAD = generalized anxiety disorder. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Figure 2.

(A) Group × condition interaction localized to left amygdala (peak MNI coordinate: -26, -4, -20). Display threshold is $p < 0.001$ whole brain uncorrected (cluster $p < 0.05$ corrected). (B) Mean parameter estimates extracted from left amygdala displayed by group and condition. (C) Group × condition interaction localized to left inferior frontal gyrus (IFG; peak Montreal Neurological Institute (MNI) coordinates: -20, 34, -16). Display threshold is $p < 0.001$ whole brain uncorrected (cluster $p < 0.05$ corrected). (D) Mean parameter estimates extracted from left IFG displayed by group and condition. Error bars reflect ± 1 standard error. HC = healthy control; GAD = generalized anxiety disorder. ** $p < 0.01$; * $p < 0.05$.

Figure 3.

(A) Graph of positive relationship between parameter estimates from left amygdala and left IFG for Reappraise > Look-Neutral and (B) Look-Negative > Look-Neutral contrasts.

Table 1.

Sample Demographics

	GAD (<i>n</i> = 30)	HC (<i>n</i> = 30)		
	<i>M</i> (<i>SD</i>)	<i>M</i> (<i>SD</i>)	<i>test</i> <i>statistic</i>	<i>p</i>
Age	27.20 (7.57)	25.43 (10.02)	0.77	0.44
Years of Education	16.43 (2.91)	14.80 (3.92)	0.41	0.07
HAM-A	14.70 (7.24)	0.87 (1.53)	10.25	< 0.001
HAM-D	10.63 (3.59)	0.73 (1.20)	14.34	< 0.001
PSWQ	66.73 (7.16)	29.37 (8.74)	18.02	< 0.001
ERT Negative Affect Ratings				
Look-Neutral	1.32 (0.44)	1.18 (0.39)	1.24	0.22
Look-Negative	3.13 (0.73)	2.46 (0.82)	3.37	0.001
Reappraise-Negative	2.83 (0.82)	2.04 (0.62)	4.18	< 0.001
	<i>n</i> (%)	<i>n</i> (%)		
Gender (Female)	18 (60.00%)	23 (76.67%)	1.93	0.16
Race (Caucasian)	23 (76.67%)	19 (63.33%)	4.99	0.17
Ethnicity (Hispanic)	3 (10.00%)	4 (13.33%)	0.16	0.69
Comorbidities				
Social Anxiety Disorder	13 (43.33%)	-	-	-
Major Depressive Disorder	6 (20.00%)	-	-	-
Panic Disorder	3 (10.00%)	-	-	-
Specific phobia	3 (10.00%)	-	-	-
Posttraumatic Stress Disorder	2 (6.67%)	-	-	-
Obsessive-compulsive Disorder	2 (6.67%)	-	-	-
None	12 (40.00%)	-	-	-
One comorbid condition	10 (33.33%)	-	-	-
More than one comorbid condition	8 (26.67%)	-	-	-

Note. HC = healthy controls; GAD = generalized anxiety disorder; HAM-A = Hamilton Anxiety Ratings Scale; HAM-D = Hamilton Depression Rating Scale; PSWQ = Penn State Worry Questionnaire; ERT = Emotion Regulation Task. Group comparisons were performed using independent *t*-test except for gender, race and ethnicity differences, which were calculated using Fisher's Exact Test due to low cell count.

Table 2.

Results of Whole Brain Analysis of Variance

Result	Brain region	Laterality	Volume (mm ³)	Z-score	peak MNI Coordinates		
					x	y	z
Main effect of Group	Middle frontal gyrus	L	2,528	4.88	-50	6	54
		L	4,848	4.70	-2	16	62
	Tuber of the cerebellum	R	3,720	4.18	38	-64	-32
	Inferior frontal gyrus	R	1,584	4.18	52	24	18
		L	4,248	3.95	-36	26	-6
		R	1,360	3.73	42	30	-14
		R	2,504	3.93	50	-60	16
	Middle temporal gyrus	R	800	3.72	52	2	-30
		L	1,536	3.55	-40	-62	20
		L	592	3.49	-20	-78	-30
Main effect of Condition	Inferior frontal gyrus	R	169,760	6.35	42	30	-14
	Cerebellum	R	129,656	6.15	14	-76	-26
	Superior temporal gyrus	R	17,416	5.12	56	-6	2
	Precentral gyrus	L	13,328	4.87	-50	-4	6
	Cingulate gyrus	L	8,664	4.20	-10	0	44
	Middle temporal gyrus	L	2,072	3.88	-58	-40	0
	Nodule of the cerebellum	Midline	784	3.73	0	-56	-38
	Parahippocampal gyrus	R	544	3.50	20	-46	-10
Group × Condition Interaction	Amygdala	L	3,640	3.93	-26	-4	-20
	Middle occipital lobe	L	1,344	3.88	-22	-86	-2
	Inferior frontal gyrus	L	1,808	3.83	-20	34	-16
	Anterior insula	L	560	3.67	-40	14	12
	Posterior insula	L	784	3.64	-42	-20	18

Note HC = healthy control; GAD = generalized anxiety disorder; MNI = Montreal Neurological Institute; L = left; R = right. Displaying results corrected for multiple comparisons across the entire brain.