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VENTROMEDIAL PREFRONTAL CORTEX REACTIVITY IS ALTERED IN GENERALIZED ANXIETY DISORDER DURING FEAR GENERALIZATION

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Background: Fear generalization is thought to contribute to the development and maintenance of anxiety symptoms and accordingly has been the focus of recent research. Previously, we reported that in healthy individuals (N = 25) neural reactivity in the insula, anterior cingulate cortex (ACC), supplementary motor area (SMA), and caudate follow a generalization gradient with a peak response to a conditioned stimulus (CS) that declines with greater perceptual dissimilarity of generalization stimuli (GS) to the CS. In contrast, reactivity in the ventromedial prefrontal cortex (vmPFC), a region linked to fear inhibition, showed an opposite response pattern. The aim of the current study was to examine whether neural responses to fear generalization differ in generalized anxiety disorder (GAD). A second aim was to examine connectivity of primary regions engaged by the generalization task in the GAD group versus healthy group, using psychophysiological interaction analysis. Methods: Thirty-two women diagnosed with GAD were scanned using the same generalization task as our healthy group. Results: Individuals with GAD exhibited a less discriminant vmPFC response pattern suggestive of deficient recruitment of vmPFC during fear inhibition. Across participants, there was enhanced anterior insula (aINS) coupling with the posterior insula, ACC, SMA, and amygdala during presentation of the CS, consistent with a modulatory role for the aINS in the execution of fear responses. Conclusions: These findings suggest that deficits in fear regulation, rather than in the excitatory response itself, are more critical to the pathophysiology of GAD in the context of fear generalization. Depression and Anxiety 00:1–9, 2012.

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Key words: fear learning; conditioning; prefrontal cortex; regulation; anxiety; GAD

INTRODUCTION

Fear generalization is the transfer of conditioned fear to perceptually similar stimuli. This process has gained interest in recent years due to its proposed role in the development and maintenance of anxiety symptoms by extending learned fear responses from threat-related stimuli to nonthreatening cues. [1] The potential effect of generalization is exemplified in PTSD, in which symptoms of the disorder can be elicited by cues/situations...
far removed from the initial trauma. Similarly, in generalized anxiety disorder (GAD), generalization may contribute to an increase in the number of cues/events capable of triggering worry—a cardinal symptom of the disorder that encompasses a broader range of topics and occurs more frequently in patients with GAD relative to healthy individuals.\cite{1,2}

Studies have begun to examine fear generalization in healthy individuals using fear-potentiated startle\cite{3,4} and skin conductance\cite{5,6} to quantify fear responses to a conditioned stimulus (CS) and generalization stimuli (GS) that vary in perceptual similarity to the CS. The typical response pattern observed in these studies is characterized by a steep linear slope, with a peak fear and physiological response to the CS that declines with increasing dissimilarity of the GS to the CS.

Individuals with panic disorder exhibit flatter fear gradients with more gradual decreases in fear response to the GS.\cite{7} In addition, enhanced generalization to conceptually similar stimuli (e.g., spider and spider web) has been shown to correlate with trait anxiety.\cite{8} Further evidence for a possible link between deficits in generalization and anxiety was provided by Hajcak et al.,\cite{9} who reported deficits in a generalization paradigm as a function of variation in the brain-derived neurotrophic factor (BDNF) genotype, which has been related to both learning and anxiety-related behaviors.

In a recent study, we extended this research by examining the neural correlates of generalization in a sample of 25 healthy women.\cite{10} Brain regions engaged by the generalization task included the insula, anterior cingulate cortex (ACC), supplementary motor area (SMA), caudate, amygdala, ventromedial prefrontal cortex (vmPFC), and the somatosensory cortex. The same regions have also been implicated in fear conditioning, which supports a common circuitry for these two processes. Furthermore, neural reactivity in the insula, ACC, SMA, and caudate showed a similar response gradient to what has been observed in healthy individuals using other psychophysiological measures of fear. Reactivity in vmPFC and somatosensory cortex, on the other hand, showed an opposite pattern with peak response to stimuli most dissimilar to the CS. These neural gradients suggest that while some brain regions track the fear response to GS and GS, the vmPFC may reflect fear inhibition.

The aim of the current study was to test whether neural gradients observed in our healthy group differ in GAD. Based on evidence for enhanced fear generalization in anxious individuals,\cite{11,12} we hypothesized that patients with GAD would demonstrate flatter slopes for neural gradients relative to healthy individuals. A second aim was to compare connectivity for the GAD and healthy groups in primary regions engaged by the generalization task. To address our second aim, we conducted psychophysiological interaction (PPI) analysis to examine coupling between the anterior insula (aINS) and the ACC, SMA, caudate, amygdala, vmPFC, and somatosensory cortex as a function of the CS and GS.

We selected the aINS as the “seed” region because of its proposed role in modulating attention, autonomic reactivity, and motor responses, and its relation to anxiety;\cite{13} this region also showed the most robust activation across participants. We hypothesized that the aINS would show greater coupling with regions implicated in attention and physiological expression of fear during the presentation of the CS. Based on findings of increased insular activation in anxious populations,\cite{14} we predicted that these PPI effects would be stronger in GAD.

**METHODS**

**PARTICIPANTS**

Thirty-two women with a diagnosis of GAD (17 with and 15 without comorbid major depression; Mean age overall = 22.3, SD = 4.5) and 25 healthy women\cite{15} (Mean age = 21.6, SD = 5.1) were included in the analysis. We recruited only women to reduce gender-related heterogeneity in the sample and because anxiety is more commonly diagnosed in females. All participants completed the Structured Clinical Interview for DSM-IV Axis I Disorders—Patient Edition, Version 2 (SCID-I/P)\cite{16} to confirm diagnoses of GAD in the patient group and absence of Axis-I diagnoses in the healthy group. None of the participants were currently using any psychotropic medications. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

**EXPERIMENTAL PARADIGM**

The experimental paradigm has been previously described in detail.\cite{17} The task consisted of 120 trials (15 trials × 8 conditions). Stimuli were seven red rectangles with identical height and varying width. A middle-sized rectangle was the CS; half of the time the CS co-terminated with an electric shock (CSpaired), whereas half of the time it did not (CSunpaired). The six remaining rectangles differed by ±20%, ±40%, or ±60% in width from the CS, and served as the GS. Stimuli were presented pseudorandomly for 2 s with a jittered interstimulus interval ranging from 4 to 10 s. Prior to scanning, participants were instructed that the middle-sized rectangle (CS) indicated a 50% probability that they would receive a subsequent electric shock, but that shocks would never follow rectangles of greater or lesser size. Previous studies demonstrate that results are comparable whether participants are informed about the CS-US contingency\cite{18,4} or learn it themselves.\cite{19} To confirm task efficacy, we assessed autonomic response to all stimuli using pupillary response measures (Eyelink-1000; SR Research Ltd., Ontario, Canada). In addition, we collected posttask ratings of shock-likelihood for each rectangle, rated on a Likert-type scale of 1 (certainly not shocked) to 5 (certainly shocked).

**SELF-REPORT QUESTIONNAIRES**

After the fMRI session, participants completed the trait scale of the State-Trait Anxiety Inventory (STAI-T)\cite{18} and the Beck Depression Inventory (BDI-II)\cite{20} to assess continuous measures of self-reported anxiety and depressive symptoms, respectively.

**IMAGE ACQUISITION**

We acquired 440 T2*-weighted echoplanar images with an oblique coronal angle and repetition time (TR) = 2,100 ms, echo time (TE) = 23 ms, flip angle = 83°, matrix = 96 × 96, field of view (FOV) = 224 × 224 mm, slices = 37 and slice thickness = 3.5 mm. In

\[1\] Results for the healthy group were previously reported in Ref. [8].
addition, we obtained T1-weighted structural scans with TR = 1,900 ms, TE = 2.53, flip angle = 9°, FOV = 176 x 250 x 250 mm and matrix = 176 x 256 x 256 mm.

**IMAGE ANALYSIS**

Preprocessing procedures were performed in SPM8 (www.fil.ion.ucl.ac.uk/spm) and included slice time correction, motion correction, normalization, and smoothing with a 6-mm full width at half-maximum Gaussian kernel. Preprocessed images were entered into a general linear model in which each rectangle was modeled as an event with no duration (CSpaired and CSunpaired were modeled separately). The six motion parameters estimated during realignment were included as regressors of no interest and serial autocorrelations were modeled using an AR (1) process.

**GRADIENTS OF NEURAL REACTIVITY**

We generated neural gradients for all regions previously examined in the healthy group, including the right and left insula, ACC, SMA, right and left caudate, vmPFC, and somatosensory area by extracting the first eigenvariate (i.e., the principal component) from a 6-mm sphere centered on the local maxima (P < .05, family-wise error corrected) within each region, for each of the “CSpaired ~ Baseline” (i.e., fixation) and “GS ~ Baseline” contrasts, across all participants. Mean values for CSunpaired, as well as GS ± 20%, GS ± 40%, and GS ± 60%, were plotted as a 4-point gradient.

**PSYCHOPHYSIOLOGICAL INTERACTION (PPI)**

PPI is a connectivity technique based on regression models; it identifies which voxels within the entire brain (or within regions of interest) show increased coupling with a seed region, in response to specific conditions of a task (see Ref. [11] for details). Here, we conducted two PPI analyses to examine connectivity of the right aINS (seed region) with the ACC, SMA, caudate, amygdala, vmPFC, and somatosensory cortex as a function of exposure to the CS relative to GS ± 60% (first analysis) and the CS relative to all GS combined (second analysis). The psychological (or treatment) vector was “CS versus GS ± 60%” (“CS versus GS ± 20% + GS ± 40% + GS ± 60%” for the second analysis) and the physiological vector, for both analyses, was the first eigenvariate time-series extracted from a 6-mm radius sphere centered on the maximally activated voxel in the right aINS for each participant using a height threshold of P < .05 and extent threshold = five contiguous voxels (the search area for the maximally activated voxel for each participant was restricted to a 6-mm radius mask centered on the peak voxel in the aINS from the main effect F-contrast of the entire sample). For seven participants (three GAD and four healthy individuals), there were no significant voxels within the seed region at this threshold and these participants were excluded from further analysis. Contrast images from all remaining participants (N = 50) representing the interaction term between the source time-series and treatment vector were used in second-level random effects analyses with a whole-brain threshold of α = .001(uncorrected) and a small volume family-wise error rate corrected α = .05 for the ACC, SMA, caudate, amygdala, vmPFC, and somatosensory cortex using individual bilateral masks generated with the Masks for Regions of Interest Analysis software.[14]

**PUPIAL DATA**

Preprocessing procedures were described elsewhere. In short, we calculated a baseline for each trial by averaging data points from 500 ms immediately preceding the onset of stimulus and then subtracting this mean from each trial. Pupillary response was defined as cumulative pupil diameter change (i.e., area under curve) within a 1,000 ms window, starting 1 s after stimulus onset. Trials were averaged by stimulus type, combining ± conditions to minimize confounding effects of stimulus-specific luminosity. Data for six participants were excluded due to technical problems.

**STATISTICAL ANALYSES FOR BEHAVIORAL MEASURES AND NEURAL GRADIENTS**

We assessed shock-likelihood ratings and pupillary response with a 2 (group: GAD versus Healthy) x 4 (stimulus type: CSpaired, GS ± 20%, GS ± 40%, GS ± 60%) mixed-model repeated-measures analysis of variance and a mixed linear model, respectively. Pairwise comparisons were made with the Bonferroni adjustment for multiple comparisons. We examined group differences in neural gradients slopes with a 2 (group: GAD versus Healthy) x 4 (stimulus type) trend analysis for repeated measures; GAD (N = 32), Healthy (N = 25). Comparisons between the GAD only (N = 15) and GAD with comorbid depression (N = 17) subgroups did not reveal significant results for these measures (all Ps > .05).

**RESULTS**

**SHOCK LIKELIHOOD AND PUPIAL RESPONSE MEASURES**

Shock-likelihood ratings varied as a function of stimulus type (F(3,165) = 108.3, P < .001; see Fig. 1). Pairwise comparisons confirmed the impression from Fig. 1 that shocks were rated as more likely following the CS (M = 4.05, SD = 1.03) compared to GS ±20% (M = 2.87, SD = 1.03; P < .001), GS ±40% (M = 1.77, SD = .88; P < .001), and GS ±60% (M = 1.3, SD = .77; P < .001). In addition, shocks were rated as more likely following the GS ±20% compared to both GS ±40% (P < .001) and GS ±60% (P < .001), and the GS ±40% compared to GS ±60% (P < .001). The main effect of group and stimulus by group interaction were not significant (P = .26 and .19, respectively).

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

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Pupillary response varied as a function of stimulus type ($F_{(3,4121.16)} = 15.59, P < .001$). Pairwise comparisons showed that pupillary response was larger for the CS unpaired versus GS ± 20% ($P < .001$), GS ± 40% ($P < .001$), and GS ± 60% ($P < .001$).

Comparisons for GS ± 20% versus GS ± 40% and GS ± 60%, and for GS ± 40% versus GS ± 60% were not significant (all $P$s $\geq .09$). The main effect of group and stimulus by group interaction were not significant (both $P$s $\geq .84$).

GRADIENTS OF NEURAL ACTIVATION

Reactivity in the insula, ACC, SMA, and caudate varied as a function of stimulus type with higher reactivity associated with increased similarity of the GS to CS (right insula: $F_{(3,165)} = 19.18, P < .001$; left insula: $F_{(3,165)} = 17.72, P < .001$; ACC: $F_{(3,165)} = 8.26, P < .001$; right SMA: $F_{(3,165)} = 20.02, P < .001$; right caudate: $F_{(3,165)} = 14.89, P < .001$; left caudate: $F_{(3,165)} = 10.59, P < .001$; neural gradients for these regions are presented in Fig. 2; gradients were similar bilaterally therefore only right-sided gradients are shown). A trend analysis for these regions did not show a significant stimulus type by group interaction (all $P$s $\geq .39$) and there was no significant main effect of group (all $P$s $\geq .35$). In contrast, reactivity in the vmPFC ($F_{(3,165)} = 15.53, P < .001$) and somatosensory cortex ($F_{(3,165)} = 11.5, P < .001$) showed a reverse response pattern, with highest response to the GS most dissimilar to the CS. A trend analysis in both regions revealed a significant stimulus type by group interaction (vmPFC: $F_{linear (1,55)} = 4.3, P < .05$; somatosensory cortex: $F_{linear (1,55)} = 30.54, P < .001$) with the GAD group showing flatter slopes across stimuli relative to the healthy group (Fig. 3).

CONTINUOUS MEASURES OF ANXIETY AND DEPRESSION

Mean STAI-T and BDI scores were higher in the GAD group (STAI-T: $M = 55.09, SD = 10.08$; BDI: $M = 22.34, SD = 12.66$) compared to the healthy group (STAI-T: $M = 37.52, SD = 6.52$; BDI: $M = 4.76, SD = 3.54$; both $P$s $< .001$).

STAI-T and BDI scores were positively correlated with slope coefficients of individual vmPFC gradients ($r = .33, P = .01$ and $r = .39, P = .003$, respectively; Fig. 4). Independent correlation analyses in the GAD and healthy groups showed that these associations were only significant for the GAD group ($P$s $< .04$).

PPI ANALYSES

Across participants, PPI results indicated that, during presentation of the CS relative to the GS ± 60%, activity...
in the right aINS showed greater covariance with activity in the posterior insula (pINS), ACC, SMA, and the amygdala (Fig. 5). For the second analysis, in which we compared connectivity during the CS relative to all GS, activity in the aINS showed greater coupling with activity in the pINS and the amygdala (Fig. 6). There were no significant PPI effects for the opposite contrasts (i.e., during presentation of the GS ± 60%, or GS combined, relative to the CS). We did not find any differences in PPI effects between the GAD group and the healthy group.

**DISCUSSION**

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

The vmPFC has been implicated in attenuation of fear responses in both animals and humans. Animal studies have shown that lesions in vmPFC regions impair extinction recall.[13,16] In humans, fMRI studies have reported correlations between vmPFC activation and magnitude of extinction memory.[17,18] Additional evidence for the role of the PFC in controlling fear comes from emotion regulation studies in which participants are instructed to re-appraise aversive stimuli in order to reduce their negative impact.[19] These studies have reported increased activation in lateral PFC during downregulation of negative images with corresponding decreases of activation in the amygdala.[20,21] A comparison of PFC function during emotion regulation and extinction suggests that during reappraisal, the lateral PFC inhibits the amygdala via a shared circuitry with extinction that involves the vmPFC.[22]

Neurocircuitry-based models of anxiety disorders have hypothesized hyperresponsivity to threat within the amygdala and deficient vmPFC function mediating inadequate regulation of fear responses.[23,24] These response patterns have been demonstrated most consistently in PTSD.[25,26] For example, compared to healthy individuals, patients with PTSD exhibit decreased activation in the vmPFC and increased activation in the amygdala in response to symptom provocation paradigms with trauma-related cues.[27,28] Reduced activation of vmPFC in PTSD has also been found during extinction recall[29,30] and was associated with symptom severity.[31,32] In GAD, findings suggest dysfunction in the amygdala-frontal circuitry as well.[33–35] However, the role of these structures in the pathophysiology of GAD remains poorly understood due to the small number of neuroimaging studies that have focused on this disorder, mixed results from existing studies, and lack of data from paradigms of fear learning.[25] Nevertheless, a recent study demonstrated that patients with GAD fail to activate the pregenual ACC (a sub-section of the vmPFC) during implicit regulation of

Figure 4. Pearson correlations between the trait scale of the State-Trait Anxiety Inventory (STAI-T) (a) and Beck Depression Inventory (BDI) (b) scores and slope coefficients of individual vmPFC gradients for the GAD group (STAI-T: $r = .37, P = .039$; BDI: $r = .42, P = .017$; unfilled circles/dotted line) and healthy group (STAI-T: $r = -.09, P = .68$; BDI: $r = -.06, P = .76$; filled circles/solid line).
The vmPFC response pattern observed in the current study suggests that recruitment of vmPFC may also be deficient in GAD when inhibition of fear responses is required (i.e., during presentation of “safe” stimuli). Across GS, the GAD group exhibited less discriminant vmPFC responses as evident by a flatter vmPFC gradient. Slope coefficients of patients’ individual vmPFC gradients were positively correlated with trait anxiety and depressive symptoms. Healthy individuals, on the other hand, showed greater increases in vmPFC reactivity to consecutive stimuli (reflected by a steeper vmPFC gradient) and their vmPFC slope coefficients were not associated with measures of anxiety and depression. A failure to properly engage the vmPFC in the presence of stimuli that resemble a CS could facilitate fear generalization and might reflect a broader dysfunction of regulatory skills in GAD, in accordance with its diagnostic criteria pertaining to difficulties in controlling worry and anxiety.

Reactivity in the somatosensory cortex was also less discriminate in the GAD group. Based on findings of deactivation in somatosensory areas when shocks were expected, but not delivered, we previously hypothesized that reactivity in this region may be associated with participants’ expectation of shocks that are not presented. Accordingly, the response pattern exhibited by the GAD group in this region may be indicative of less certainty regarding stimulus-shock contingencies. Although posttask ratings of shock likelihood were similar for the two groups, this interpretation is supported by greater variability in scores for the GAD group.2

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

The GAD and healthy group did not differ in insular connectivity with the ACC, SMA, and amygdala.

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2Levene’s test for equality of variances between the two groups was significant for the GS ± 60% (P < .001) and GS ± 40% (P = .02).
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Figure 6. Psychophysiological interactions for the right anterior insula (aINS) seed during presentation of the CS relative to all GS (i.e., GS ± 20%, GS ± 40%, and GS ± 60%). (a) Right aINS seed. (b) Positive connectivity with the left amygdala (lAmy).

during presentation of the CS. Reactivity in these regions, as well as pupillary responses across stimuli, was also the same for the two groups. These results indicate that the strength of the fear response, and its underlying circuitry, were comparable in patients and healthy individuals.

Because participants in this study were young with a relatively short history of symptoms, future studies should examine whether GAD patients with longer illness durations present other alterations in neural reactivity during fear generalization. The current study, like most studies of fear generalization, utilized GS that changed along a physically neutral stimulus dimension (i.e., size). Dunsmoor et al. [5] proposed that fear-relevant attributes of the GS (e.g., intensity of fear expression) may increase generalization. Neural gradients to such stimuli, including disorder-specific GS, may better distinguish anxious patients and healthy individuals.

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

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