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# Uncertainty Potentiates Neural and Cardiac Responses to Visual Stimuli in Anxiety Disorders

Jaryd Hiser, Brett Schneider, and Michael Koenigs

**ABSTRACT**

**BACKGROUND:** Intolerance of uncertainty and worry about future events are cardinal features of anxiety. However, the neurobiological and physiological mechanisms underlying these characteristics of anxiety remain to be fully elucidated.

**METHODS:** Individuals with diagnosed anxiety disorders ($n$ = 29, 22 female) and age-matched comparison subjects ($n$ = 28, 17 female) completed a task in which pictures (aversive or neutral content) were preceded by cues indicating certainty or uncertainty about the emotional valence of the subsequent pictures. We assessed functional magnetic resonance imaging and heart rate activity with respect to the 1) cue period, 2) emotional valence of the pictures, and 3) modulatory effect of uncertainty on responses to subsequent pictures.

**RESULTS:** Individuals with anxiety disorders and comparison subjects exhibited similar functional magnetic resonance imaging and cardiac activity during the cue period and for the aversive versus neutral picture contrast. However, individuals with anxiety disorders exhibited greater modulatory effects of uncertainty on their responses to subsequent pictures. Specifically, they displayed greater functional magnetic resonance imaging activity in a number of cortical regions (visual cortex, anterior cingulate cortex, superior temporal gyrus, and anterior insula), as well as significantly reduced cardiac deceleration to pictures preceded by the uncertainty cue.

**CONCLUSIONS:** These findings suggest that heightened neural and autonomic reactivity to stimuli during conditions of uncertainty may be a key psychobiological mechanism of anxiety.

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Anxiety disorders are the most common form of psychopathology, with a lifetime prevalence of approximately 30% in the United States (1). The total annual cost of anxiety disorders has been estimated to be approximately $40 billion (2,3). These costs are mostly attributed to morbidity, mortality, lost productivity, and other indirect costs (4). Most individuals with anxiety disorders do not present with a single disorder. There is high comorbidity between anxiety disorders and other anxiety disorders, depressive disorders, substance use disorders, and/or personality disorders (5). Individuals with anxiety disorders have suboptimal rates of recovery (approximately 58% over a 12-year period for individuals receiving treatment) and in cases where patients do recover, there are significant recurrence rates (45%) (6,7). Both cognitive behavioral psychotherapy and psychopharmacological treatments have been found to be effective in the treatment of anxiety (8), but symptom reduction and remission remains a problem.

One avenue of research that may enhance efficacy of anxiety diagnosis and treatment is elucidating the neurobiological and psychological mechanisms underlying anxiety. With a deeper understanding of these psychobiological mechanisms, we can hopefully increase our ability to assess and treat anxiety disorders effectively and efficiently. For example, the identification of reliable neuroimaging or physiological markers could be used to develop more objective diagnoses including psychobiological subtypes, predict course of illness or the efficacy of different treatment options, and identify potential anatomical targets for interventions such as neurostimulation techniques. In this study, we use a combination of functional neuroimaging and peripheral physiology measures to probe three putative neuropsychological mechanisms underlying anxiety disorders.

One psychological feature that is thought to play a central role in anxiety is intolerance of uncertainty (IU)—the distress from the possibility that negative events may occur unpredictably (9–11). It has been suggested that a common feature across anxiety disorders is aberrant and excessive anticipatory responding under conditions of threat uncertainty (9). Prior work has also suggested that IU is a broad specifier for worry, which is a cognitive strategy that individuals use in an attempt to control the unknown (12,13). Moreover, the network of brain regions that become active during the anticipation of uncertain outcomes (e.g., anterior insula, amygdala, ventromedial prefrontal cortex [vmPFC], and anterior cingulate cortex [ACC]) (14–18) has also been widely implicated in the pathogenesis of anxiety disorders (9,19–23).

A second feature that may play a role in anxiety is the intensity of emotional responses to negative events. According to the emotion dysregulation model of anxiety (24), individuals with anxiety experience heightened intensity of emotions, poor

 

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understanding of their emotions, and deficient emotion regulation. It has been suggested that individuals with anxiety have emotional responses that occur more easily, quickly, and intensely than those without anxiety (25). In addition, previous studies have shown that individuals with anxiety display maladaptive peripheral physiological responses during emotional contexts [i.e., reduced heart rate variability (26,27) and reduced cardiac deceleration, indicating a shift to cardiac acceleration and potentiated, defensive responding (28)]. Despite these theories suggesting emotional hyperreactivity in anxiety disorders, evidence from prior neuroimaging studies examining the neurobiological correlates of emotional hyperreactivity in anxiety disorders remains mixed (29–31).

A third feature that may play a role in anxiety disorders is the modulation of emotional responses by uncertainty. Clinically, individuals with anxiety often worry about the possibility of an aversive stimulus, which can lead to an exaggerated emotional response regardless of whether the aversive stimulus is present or not. Previous research has shown that in healthy subjects, aversive events elicit heightened subjective and physiological responses when preceded by uncertainty (14,32–34). Previous research has also shown that exposure to unpredictable neutral tones elicits greater amygdala activity and anxious behavior than predictably timed tones, indicating that uncertainty can modulate subsequent stimulus-evoked responses (35). However, to date, no research has examined the effect of uncertainty on subsequent stimulus response in a population of individuals with anxiety disorders.

In this study, we addressed this empirical gap through an application of functional magnetic resonance imaging (fMRI) and heart rate responses in individuals with anxiety disorders. Our aim was to examine neural and cardiac activity during uncertainty and in response to emotional stimuli separately, as well as the effect of uncertainty on subsequent responses to visual stimuli. To address these aims, we used a paradigm previously shown to elicit neural response to aversive pictures and uncertain cues in healthy subjects (14). Specifically, we hypothesized that for individuals with anxiety disorders as compared with comparison subjects, 1) uncertainty during the anticipation of aversive or neutral stimuli would be associated with greater activity in the anterior insula and amygdala, as well as potentiated cardiac response (i.e., reduced heart rate deceleration); 2) viewing aversive relative to neutral images would be associated with greater activity in the amygdala, less activity in the vmPFC, and potentiated cardiac response; and 3) the modulatory effect of uncertainty on responses to subsequent stimuli would be associated with greater activity in the anterior insula and amygdala and potentiated cardiac response.

## METHODS AND MATERIALS

### Participants

This study was approved by the University of Wisconsin-Madison Institutional Review Board; all subjects provided written informed consent. Psychiatric diagnoses were based on DSM-5, through the Structured Clinical Interview for DSM-5 (SCID) (36). Subjects were eligible to be included in the individuals with anxiety group ($n$ = 34) if they were 18 years or older, had a primary diagnosis of generalized anxiety disorder (GAD) (based on DSM-5 criteria), and were free of psychotropic medications for 6 weeks before study enrollment. Exclusion criteria were history of significant brain injury, neurological disorder, psychosis, bipolar disorder, posttraumatic stress disorder, or moderate or severe substance use disorder. In addition, healthy comparison (HC) subjects ($n$ = 34) with no history of brain injury, neurologic or psychiatric illness, current use of psychoactive medication, or history of psychotherapy treatment were recruited. Six participants from the HC group and 5 from the anxiety group were removed from the analysis because of excessive motion during the experiment (>10% of volumes censored), resulting in a final sample of 57 subjects ($n$ = 28 HC and $n$ = 29 anxiety). Owing to the high rate of comorbidity among anxiety disorders (1), comorbid mood and anxiety disorders were permitted. In the individuals with anxiety disorders group, 6 participants had comorbid major depression, 14 had social anxiety disorder, 5 had obsessive-compulsive disorder, 9 had phobic disorder, and 17 had panic disorder. Demographic and neuropsychological data for the anxiety and HC groups are summarized in Tables S1 and S2.

### Experimental Design

Before scanning, subjects were informed of all cue-picture contingencies and completed a practice task consisting of 16 unique trials (4 per cue-picture pair) to ensure task comprehension. During the fMRI task, which was adapted from previous studies (14,37–39), subjects viewed 64 unique images drawn from the International Affective Picture System (40), divided evenly among pictures with aversive or neutral content. Aversive stimuli consisted of 32 aversive/unpleasant and arousing images, based on published norms (40,41). Neutral stimuli consisted of 32 images with neutral valence and low arousal ratings. Further information about the stimuli used can be found in Table S3. All images were preceded by one of three visual cues (“X,” “O,” or “?”). The X and O cues indicated that the subsequent image would be aversive or neutral, respectively, whereas the ? cue provided no information regarding the emotional content of the image (equal likelihood of aversive or neutral content). Thus, aversive (X) and neutral (O) cues predicted certain outcomes, whereas ambiguous (?) cues indicated uncertainty about the impending stimulus. Each experimental trial consisted of a cue presented for 2 seconds, followed, after a jittered interstimulus interval (ISI; range: 2–8 s), by a 1-second picture presentation. After a second jittered ISI (range: 5–9 s), subjects had 4 seconds to rate their emotional response to the image using a 4-item scale ranging from 1 (“very positive”) to 4 (“very negative”). See Figure S1 for a schematic diagram of the paradigm.

### MRI Data Acquisition

The MRI data acquisition details are described in the Supplement.

### Statistical Analyses

Data analysis was conducted using AFNI (42). Individual task runs were slice time corrected, motion corrected, smoothed with a 6-mm full width at half maximum Gaussian kernel, and scaled to percentage signal change (PSC). Preprocessed task

data were concatenated and analyzed as previously described to separately model phasic and sustained components of anticipatory activity (39). Phasic activity was modeled using stick regressors at the onset of each cue, and sustained activity was modeled using a duration-modulated boxcar regressor, beginning at cue offset and spanning the 2–8 seconds of anticipatory ISI. All six cue regressors (three phasic and three sustained) were included in a general linear model with additional regressors for each picture type; a single regressor for the rating period; and several regressors of no interest, including six motion covariates from rigid body alignment (43) and a fourth-order polynomial to model baseline and slow signal drift. Blood oxygen level–dependent signal was modeled by convolving each regressor with AFNI's default canonical hemodynamic response function (gamma function). To avoid potential confounds introduced by subject motion, volumes in which 10% of voxels were time-series outliers were censored before conducting the general linear model; there are no group differences in the average proportion of censored volumes ($p$ = .41) or in mean framewise displacement ($p$ = .20). Resulting whole-brain maps of voxelwise values for phasic and sustained responses were aligned to Montreal Neurological Institute space and resampled to 3 $mm^3$ isotropic resolution for second-level analyses.

To identify brain regions in which cue-related activity, picture response activity, and the modulatory effect of uncertainty on responses to subsequent stimuli differed between groups, we performed three between-group $t$ tests. The picture response model includes valence (aversive vs. neutral) and group (HC vs. anxiety), as well as covariates for age and gender. The cue-related model included cue (?, X, O), group (HC vs. anxiety), as well as covariates for age and gender. Finally, the modulation model included pictures preceded by uncertain cues versus pictures preceded by certain cues and group (HC vs. anxiety), as well as covariates for age and gender. Resulting statistical maps were familywise error (FWE)–corrected for multiple comparisons across the whole brain at the cluster level ($p_{FWE} < .05$), using a height threshold of $p < .002$ (44,45). A corrected $p_{FWE} < .05$ was achieved using a cluster extent threshold of 15 contiguous voxels, calculated using Monte Carlo simulations with 3dClustSim in AFNI. In addition to whole-brain analyses, we also performed region of interest (ROI)–based analyses for each between-group $t$ test using anatomically derived ROIs for a group mask including the vmPFC, bilateral amygdala, and bilateral anterior insula (Figure S2). For ROI-based analyses, a corrected $p_{FWE} < .05$ was achieved using a cluster extent threshold of six contiguous voxels within the ROI, calculated using Monte Carlo simulations with 3dClustSim in AFNI.

To assess cardiac responses, we analyzed cardiac plethysmography data to compute trialwise estimates of heart rate change for each subject, as previously described (28). Cardiac R-spikes were identified using interactive beat detection software. Trials with ectopic beats, missed beats, or periods of noisy signal (where beat detection failed) were excluded from further analysis (HC group, $n$ = 4 with one excluded trial; anxiety group, $n$ = 3 with one excluded trial). R-R intervals were transformed into heart rate in beats per minute, averaged in 500-ms bins. Changes in heart rate were determined by subtracting the mean heart rate for 1 second preceding each stimulus (picture or cue) from the heart rate over 7 seconds after stimulus onset (in 500-ms bins). As in previous studies, the maximum cardiac deceleration (i.e., heart rate decrease) during the first 3 seconds of stimulus onset was used as an index of the physiologic response to each picture (28).

## RESULTS

### Behavioral Data

During the fMRI task, both groups rated aversive pictures as significantly more aversive than neutral pictures, with no significant differences between groups in ratings ($p > .43$) or reaction times ($p > .17$). There were no significant differences (within or between groups) for pictures that were preceded by uncertain or certain cues in ratings ($p > .38$) or reaction times ($p > .31$). See Supplemental Results and Table S3 for full behavioral data.

### fMRI Data: Response to Certain/Uncertain Cues

To test the hypothesis that individuals with anxiety disorders would have elevated activity in the anterior insula and amygdala during the uncertain anticipation period (relative to HC subjects), we conducted whole-brain and ROI-based between-group $t$ tests on PSC in response to uncertain versus certain cues. Contrary to our hypothesis, there were no significant group differences (whole-brain or ROI based) in neural response to uncertain versus certain cues.

### fMRI Data: Response to Aversive/Neutral Pictures

Consistent with previous studies, aversive relative to neutral pictures (regardless of preceding cue type) elicited robust subcortical and visual cortex activation in HC subjects and individuals with anxiety disorders (46–48) (Figure S3). To test the hypothesis that individuals with anxiety disorders would have elevated amygdala responses and decreased vmPFC responses to aversive pictures (relative to HC subjects), we conducted whole-brain and ROI-based between-group $t$ tests on PSC in response to aversive versus neutral pictures. Again, contrary to our hypothesis, there were no significant group differences (whole-brain or ROI based) in neural response to aversive versus neutral pictures.

### fMRI Data: Modulatory Effect of Cues on Response to Pictures

To test the hypothesis that the modulatory effect of uncertainty on responses to stimuli would be associated with greater activity in the anterior insula and amygdala in individuals with anxiety disorders (relative to comparison subjects), we conducted whole-brain and ROI-based between-group $t$ tests on PSC in response to pictures preceded by uncertain cues versus pictures preceded by certain cues. The whole-brain analysis revealed significantly greater activity in the individuals with anxiety group in a number of cortical regions, including the right visual cortex, right ACC, and superior temporal gyrus (STG) (Table 1 and Figures 1 and 2). In addition, to confirm that this effect was driven by a heightened response to pictures preceded by uncertain cues, we examined both trial types separately (Figure S4). The ROI-based analysis revealed significantly greater activity for individuals with anxiety

**Table 1. Cluster Maxima for Regions With Statistically Significant Increased BOLD Signal for Whole-Brain Group Difference Between Pictures Preceded by Uncertain Cues Relative to Pictures Preceded by Certain Cues**

| | Cluster | | Peak Voxel | | | |
|---|---|---|---|---|---|---|
| Brain Region | BA | Size | *t* | x | y | z |
| R Visual Cortex | 30 | 86 | 4.63 | 15.8 | −57 | 3 |
| R Superior Temporal Gyrus | 22 | 59 | 4.36 | −61.2 | 39.5 | 17 |
| R Anterior Cingulate Cortex | 32 | 22 | 4.00 | −15.8 | −34 | 6.5 |
| R Superior Temporal Gyrus | 21 | 21 | 4.34 | −61.2 | 1 | −7.5 |

Clusters ordered by *t* score for the group difference of pictures preceded by uncertain cues > pictures preceded by certain cues contrast. Corrected *p* thresholds indicate minimum FWE-corrected *p* value for each cluster.

BA, Brodmann area; BOLD, blood oxygen level–dependent; FWE, familywise error; R, right.

disorders in the right anterior insula, however, only when examining response to aversive images (Table 2 and Figure 3). The whole-brain analysis revealed trending bilateral anterior insula activation but did not meet statistical significance (i.e., these clusters survive at a threshold of $p = .0025$, but not $p = .002$).

## Heart Rate Data: Response to Certain/Uncertain Cues

To test the hypothesis that individuals with anxiety disorders would have potentiated cardiac responses (i.e., less heart rate deceleration indicating a shift toward heart rate acceleration) during the uncertain anticipation period (relative to HC subjects), we conducted between-group *t* tests on cardiac deceleration in response to uncertain versus certain cues. Contrary to our hypothesis, there were no significant group differences in cardiac deceleration to uncertain versus certain cues.

## Heart Rate Data: Response to Aversive/Neutral Pictures

To test the hypothesis that individuals with anxiety disorders would have potentiated cardiac responses to aversive pictures (relative to HC subjects), we conducted between-group *t* tests on cardiac deceleration in response to aversive versus neutral pictures. Again, contrary to our hypothesis, there were no significant group differences in cardiac deceleration to aversive versus neutral pictures.

## Heart Rate Data: Modulatory Effect of Cues on Response to Pictures

To test the hypothesis that the modulatory effect of uncertainty on responses to stimuli would be associated with potentiated cardiac responses in individuals with anxiety disorders (relative to comparison subjects), we conducted between-group *t* tests on cardiac deceleration in response to pictures preceded by uncertain cues versus pictures preceded by certain cues. The magnitude of stimulus-evoked cardiac deceleration was significantly lower in individuals with anxiety disorders than in the comparison subjects for pictures preceded by an uncertain cue, relative to pictures preceded by a certain cue (HC, −1.03 [1.09]; 95% confidence interval [CI], −1.46 to −0.58; anxiety disorder group, −0.26 [0.82]; 95% CI, −0.58 to 0.5; $t = -2.94$, $p < .01$, effect size = 0.8) (Figure 4).

## Follow-up Analyses

To determine whether the significant group differences in the modulatory effect of cues on response to pictures varied with respect to the valence of the picture (neutral vs. aversive), we conducted whole-brain between-group *t* tests on PSC in response to both aversive and neutral pictures (i.e., preceded by uncertain cues vs. aversive pictures preceded by certain cues) separately. These separate whole-brain analyses for each valence revealed significant differences in a number of the same cortical regions as the main analysis, including the right visual cortex and right ACC (Figure S5), thus indicating that the modulation effect was present regardless of picture valence. To determine whether the observed group differences in fMRI activity were correlated with the observed group differences in heart rate deceleration for the modulatory effect of cues on response to pictures, we computed correlations across all subjects and within each group (individuals with anxiety disorders and HC subjects) separately for each cluster. For visual cortex, right STG, and right anterior insula clusters, larger fMRI modulation effects were associated with larger heart rate modulation effects (across the entire sample and within the individuals with anxiety disorders group for the visual cortex), indicating that, overall, heightened neural responses

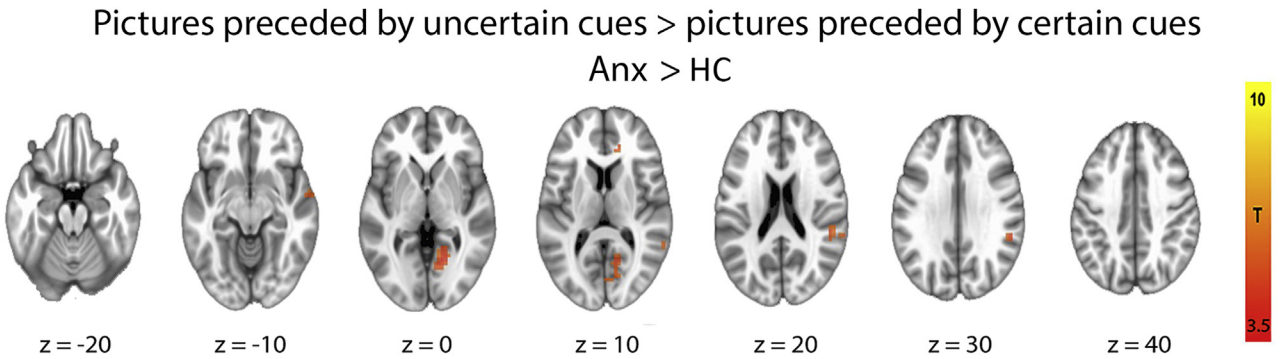


**Figure 1.** Whole-brain group difference in neural responses to pictures preceded by uncertain cue > pictures preceded by certain cue (anxiety > healthy comparison [HC] subjects; $p_{FWE} < .05$). Table 1 contains full cluster list. Anx, individuals with anxiety disorders; FWE, familywise error.

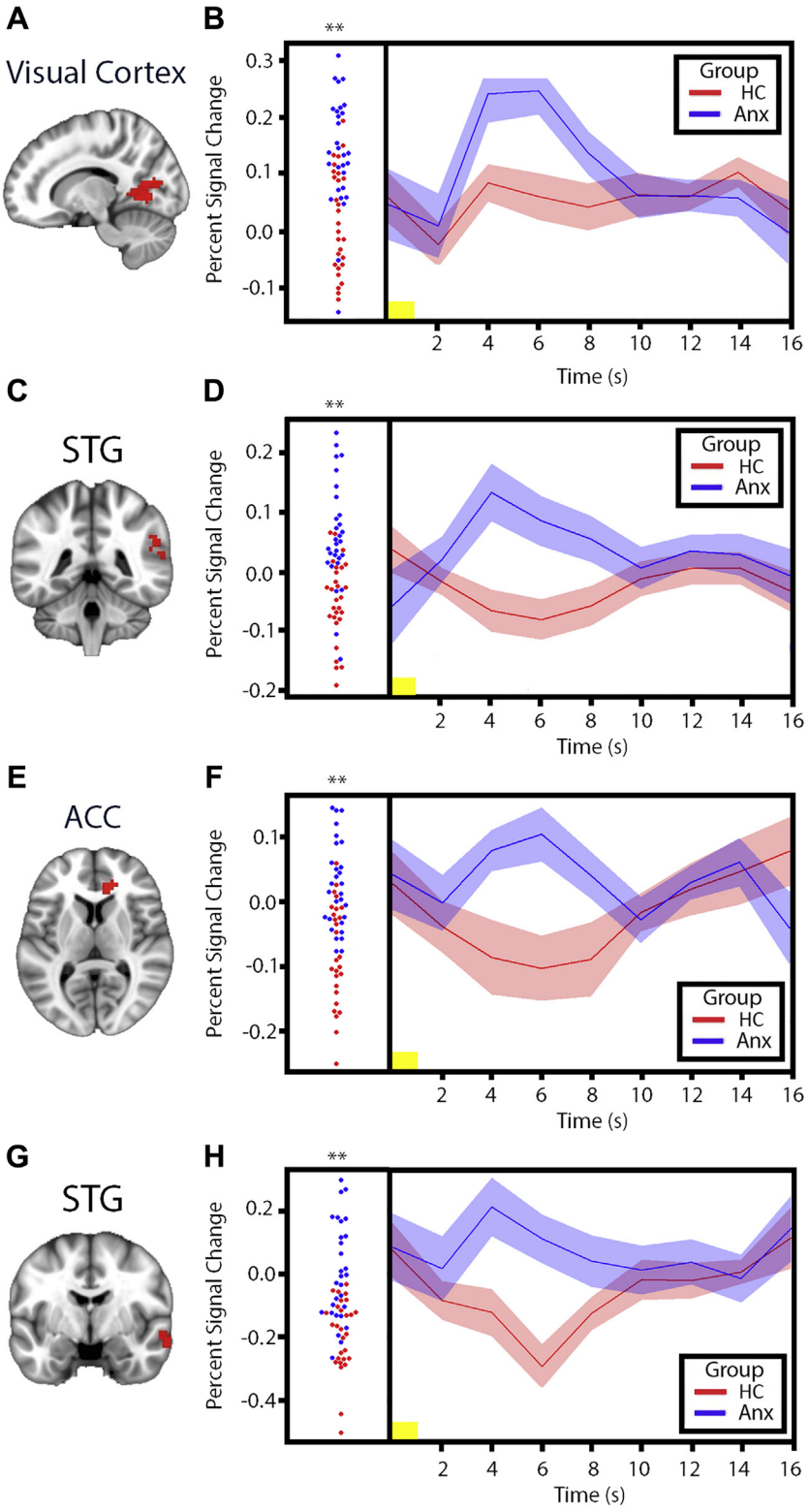


**Figure 2.** Visual cortex, superior temporal gyrus (STG), and anterior cingulate cortex (ACC) responses to pictures preceded by uncertain cues > pictures preceded by certain cues. **(A)** Task-derived visual cortex region of interest (ROI) (red) used to extract mean percent signal change (PSC) estimates for group comparison. **(B)** Left, plots of visual cortex PSC for individual healthy comparison (HC) subjects (red circles) and individuals with anxiety disorders (Anx) (blue circles) in response to pictures preceded by uncertain cues > pictures preceded by certain cues. Right, mean time series of visual cortex PSC in response to pictures preceded by uncertain cues > pictures preceded by certain cues for HC subjects (red line) and individuals with anxiety disorders (blue line) (width of the shaded area corresponds to ±1 SEM). **(C)** Task-derived STG ROI (red) used to extract mean PSC estimates for group comparison. **(D)** Plot and mean time series of PSC extracted from the STG ROI. **(E)** Task-derived right ACC ROI (red) used to extract mean PSC estimates for group comparison. **(F)** Plot and mean time series of PSC extracted from the ACC ROI. **(G)** Task-derived STG ROI (red) used to extract mean PSC estimates for group comparison. **(H)** Plot and mean time series of PSC extracted from the STG ROI. Yellow horizontal bars on the time-series plots indicate picture duration (1 s). $^{**}p < .01$.

**Table 2. Cluster Maxima for Regions With Statistically Significant Increased BOLD Signal for ROI-Based Group Difference Between Aversive Pictures Preceded by Uncertain Cues Relative to Aversive Pictures Preceded by Certain Cues**

| | Cluster | | Peak Voxel | | | |
|---|---|---|---|---|---|---|
| Brain Region | BA | Size | *t* | x | y | z |
| R Anterior Insula | 13 | 7 | 4.22 | 33.2 | 22 | 13.5 |

Clusters ordered by *t* score for the group difference of pictures preceded by uncertain cues > pictures preceded by certain cues contrast. Corrected *p* thresholds indicate minimum FWE-corrected *p* value for each cluster.

BA, Brodmann area; BOLD, blood oxygen level–dependent; FWE, familywise error; R, right; ROI, region of interest.

were accompanied by reduced cardiac deceleration in response to pictures during periods of uncertainty (Figure 5) (visual cortex: $r_{HC\text{-}Only}$ = .03, $p$ = .88, $r_{GAD\text{-}Only}$ = .54, $p < .01$, $r_{FULL}$ = .34, $p$ = .01 [$r_{FULL}$ 95% CI, 0.09 to 0.55]; right STG: $r_{HC\text{-}Only}$ = .23, $p$ = .24, $r_{GAD\text{-}Only}$ = .14, $p$ = .46, $r_{FULL}$ = .32, $p$ = .01 [$r_{FULL}$ 95% CI, 0.07 to 0.53]). No such relationship is present for the other significant fMRI clusters, although the relationship for the ACC is trending toward significance in the entire sample ($r_{FULL}$ = .24, $p$ = .07).

In sum, individuals with anxiety disorders had significantly greater neural activation in a number of regions (right visual cortex, right ACC, STG, and anterior insula) as well as significantly lower stimulus-evoked cardiac deceleration in response to the modulatory effect of cues on the response to pictures, but not to cues or pictures alone.

## DISCUSSION

In this study, neural activity (fMRI) and peripheral physiological (heart rate) data converge to demonstrate a novel psychobiological correlate of anxiety: potentiated stimulus-evoked responses in the context of uncertainty. Individuals with anxiety disorders had significantly heightened neural responses in a host of cortical brain regions—including the visual cortex, ACC, STG, and within an anterior insula ROI—in response to pictures preceded by uncertainty. Similarly, individuals with anxiety disorders had significantly reduced cardiac deceleration in response to pictures preceded by uncertainty. Here, we discuss each of these main findings.

Two of the brain regions identified in this analysis—the ACC and anterior insula—are known to be highly functionally interconnected, as the major hubs of a salience network that mediates attentional control (49,50). Moreover, both regions have been linked to anxiety (51–55) and the processing of uncertainty in comparison subjects (14–16,56,57). These results connect the previous findings on attention, anxiety, and uncertainty by demonstrating enhanced activation in these brain regions in individuals with anxiety disorders for stimuli preceded by uncertainty. This analysis also revealed greater activity in the STG and right visual cortex among the individuals with anxiety disorders. Previous fMRI studies have found abnormal activity and functional connectivity (58) and structural differences in the STG (59–61). The visual cortex, along with the amygdala and pulvinar nucleus of the thalamus, has been hypothesized to play an important role in the feedback loops that support allocation of attention to salient or significant stimuli (62). Collectively, the observed pattern of activation thus suggests enhanced neural mechanisms of attentional processing to stimuli preceded by uncertainty in individuals with anxiety disorders.

Complementing the fMRI findings, we also found that individuals with anxiety disorders had significantly reduced cardiac deceleration in response to pictures preceded by uncertainty. Reduced cardiac deceleration in response to emotional stimuli is a well-established metric of physiological defensive motivation (28). A shift to cardiac acceleration during emotional picture viewing has been suggested to indicate a high level of defense activation (28). For example, previous research has shown this shifting effect when patients with phobic disorder view pictures of the phobic object (63). Consistent with this line of work, individuals with anxiety disorders in our study had significantly reduced cardiac deceleration in response to pictures preceded by uncertainty, which may indicate a shift to cardiac acceleration. This finding suggests high levels of defense activation in response to stimuli presented in the context of uncertainty. Taken together, our

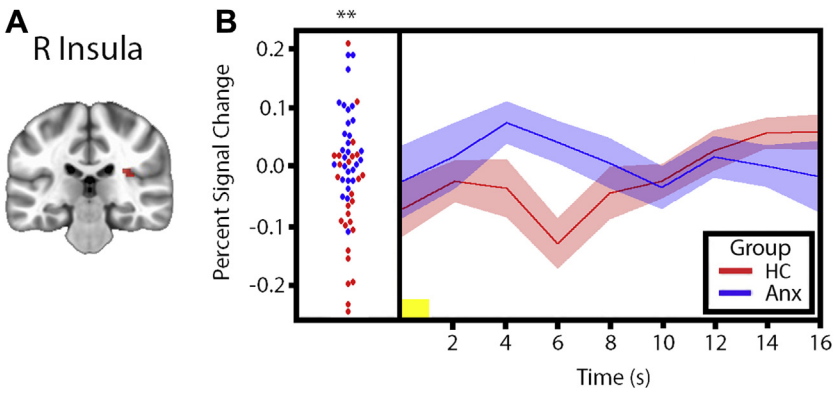


**Figure 3.** Right (R) anterior insula responses to pictures preceded by uncertain cues > pictures preceded by certain cues from region of interest–based analysis. **(A)** Task-derived visual cortex region of interest (red) used to extract mean percent signal change estimates for group comparison. **(B)** Left, plots of right anterior insula percent signal change for individual healthy comparison (HC) subjects (red circles) and individuals with anxiety disorders (Anx) (blue circles) in response to pictures preceded by uncertain cues > pictures preceded by certain cues. Right, mean time series of right anterior insula percent signal change in response to pictures preceded by uncertain cues > pictures preceded by certain cues for HC subjects (red line) and individuals with anxiety disorders (blue line) (width of the shaded area corresponds to ±1 SEM). Yellow horizontal bars on the time-series plots indicate picture duration (1 s). $^{**}p < .01$.

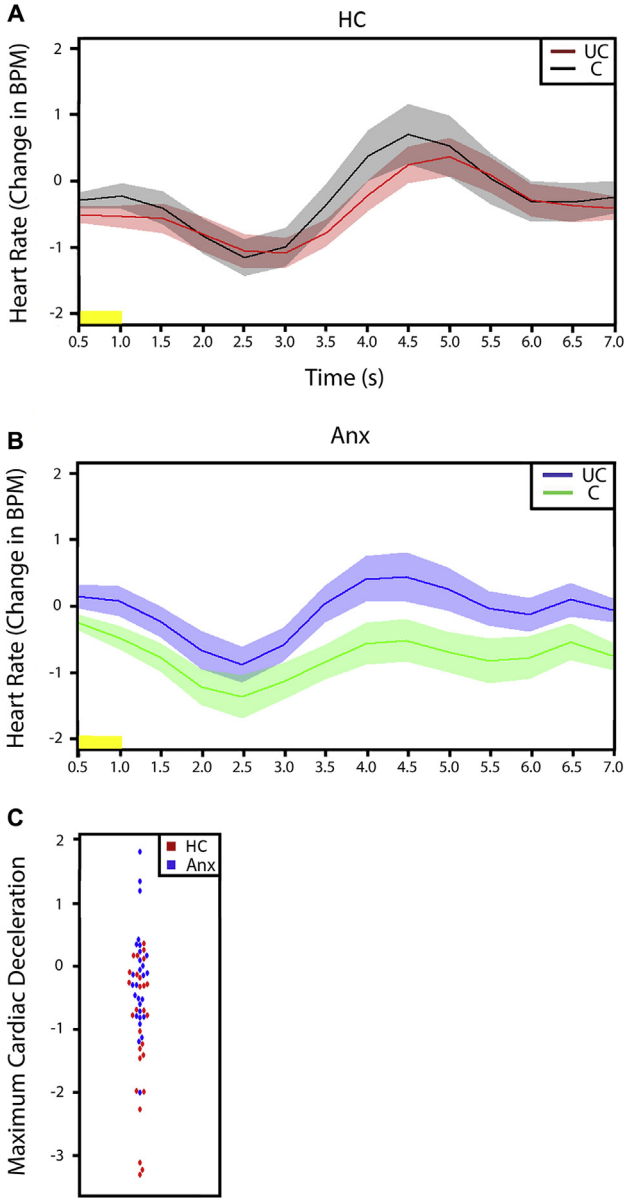


**Figure 4.** Stimulus-evoked reductions in heart rate (cardiac deceleration) in response to pictures preceded by uncertain cue (UC) and pictures preceded by certain cue (C). **(A)** Healthy comparison (HC) group. **(B)** Individuals with anxiety disorders (Anx) group. **(C)** Plot of the maximum cardiac deceleration in response to pictures preceded by UC > pictures preceded by C. Our analysis revealed significant group differences in cardiac deceleration in response to pictures preceded by UC > pictures preceded by C. Yellow horizontal bars on the time-series plots indicate picture duration (1 s). Width of the shaded area corresponds to ±1 SEM. BPM, beats per minute.

neural activity (fMRI) and peripheral physiological (heart rate) data provide complementary evidence of group differences in the modulatory effect of uncertainty on response to pictures. These findings appear in line with other recent literature that has suggested an association between higher IU and greater threat generalization (9,64,65). Therefore, based on the previous literature and the findings we present here, we propose that heightened cardiac and neural reactivity following threat uncertainty may be a key psychobiological mechanism of anxiety.

Contrary to our hypotheses, we found inconclusive results strictly in response to the cues (certain vs. uncertain) or pictures (neutral vs. aversive). To test whether our null findings were due to insufficient power, we conducted a power analysis based on effect sizes extracted from a priori ROIs. The effect sizes (range: 0.25–0.34) extracted from these ROIs would require a sample size of more than 200 participants per group to yield a statistically significant result. In addition, a recent meta-analysis (66) indicates that among neuroimaging studies of individuals with anxiety disorders, the median number of individuals with anxiety disorders sample size has been 17. In fact, of 30 such studies, only 3 (10%) have had sample sizes larger than the sample size in this study ($n$ = 29 individuals with anxiety disorders). Thus, our sample size exceeds the standard in this area of research.

Neurobiological models of anxiety disorders have suggested a key role of uncertainty in the pathogenesis of anxiety disorders (9–11). For example, IU scores are elevated in GAD (67), social anxiety disorder (68), and obsessive-compulsive disorder (68). However, in this study and other previous studies, no group differences were found in response to uncertainty alone [see (69) for a review of mixed results]. These results suggest a more complex role for uncertainty processing in the pathogenesis of anxiety disorders.

Other models of anxiety have proposed hyperactivity to threat within the amygdala and deficient vmPFC function leading to inadequate regulation of fear response (70,71). For example, previous studies of emotional anticipation (22) and emotion regulation (30) in GAD have demonstrated amygdala hyperactivity. Nevertheless, in this study and other previous studies (29–31), no group differences in response to emotional pictures were found between individuals with anxiety disorders and comparison subjects, thus challenging neuropsychological models that emphasize a primary role for the amygdala/vmPFC and threat hyperreactivity in anxiety disorders.

In conclusion, the study results suggest novel neural and physiological correlates of anxiety disorders in the modulatory effect of uncertainty on responses to stimuli. Individuals with anxiety disorders had significantly heightened neural responses in multiple brain regions involved in attentional control and significantly reduced cardiac deceleration in response to pictures following periods of uncertainty. By contrast, neural and cardiac responses were similar between groups for responses to the cues or pictures alone. Our findings thus provide unique evidence regarding the psychobiological mechanisms underlying anxiety disorders.

## Limitations

While this study examined the response of individuals with anxiety disorder to uncertainty, negatively valenced images, and the modulatory effect of uncertainty on responses to subsequent pictures, there remain several limitations. Future studies could expand the scope of these findings by using more diverse or individual specifically tailored stimuli and/or

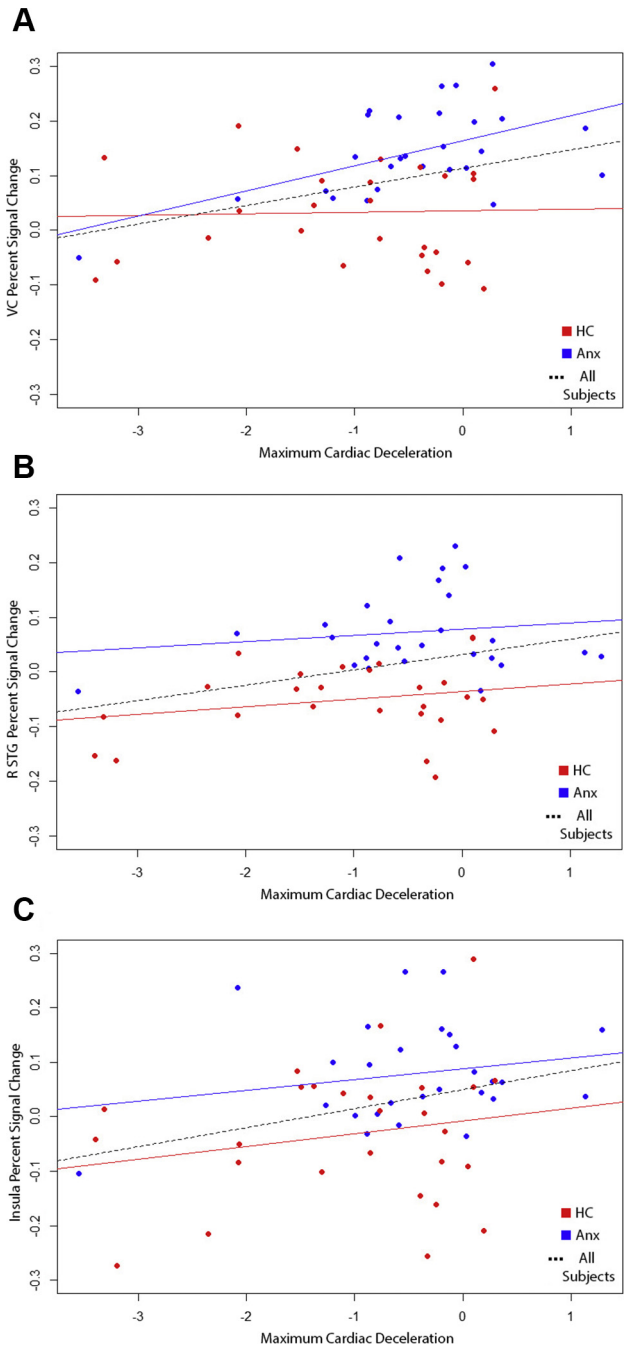


**Figure 5.** Scatter plots depicting the relationship between heart rate (cardiac deceleration) and percent signal change estimates for the **(A)** visual cortex (VC), **(B)** right (R) superior temporal gyrus (STG), and **(C)** R anterior insula in response to pictures preceded by uncertain cues > pictures preceded by certain cues. Blue dots represent individuals with anxiety disorders (Anx), and red dots represent healthy comparison (HC) subjects. Solid blue lines indicate the regression line for the 29 individuals with anxiety disorders, solid red lines indicate the regression line for the 29 HC subjects, and dotted black lines indicate the regression line across all HC subjects and individuals with anxiety disorders.

task paradigms. In addition, the use of further psychophysiology measures (i.e., skin conductance or eye tracking) could be helpful to validate these results. Recent studies have used eye tracking to monitor avoidance behaviors during experimental paradigms (72). Similarly, as we know clinically, individuals with anxiety disorders have varying levels of learned coping skills to deal with anxiety. In this study, and many others, researchers typically expect participants to attend to the stimuli, but future research should examine whether or not individuals are using coping skills to reduce anxiety during the task (i.e., emotion regulation, distraction, reassurance). As described earlier, anxiety disorder symptoms are often unique to the individual fears, and by simply using negatively valenced images, we may not capture a true anxiety response if this set of pictures does not fall within the participants' core fears. Ideally, we would be able to increase the sample size to allow us to examine how specific symptoms and/or diagnoses play an integral role in the response to uncertainty. In addition, this paradigm elicits one part of an extremely complex presentation of symptoms that combine to form psychopathology. Moreover, we did not measure IU in our sample, which varies within patients with anxiety disorder (68). A measure of IU could have been used to examine neural/cardiac responding in a continuous way instead of relying on grouping.

## Future Directions and Clinical Implications

We believe it will be important to replicate and extend these findings by addressing the specific limitations of this study that we mentioned previously. This could be achieved by taking several steps. First, it would be important to expand the sample size to examine how specific symptom clusters of anxiety disorders may alter response during periods of uncertainty. It would also be important to individualize stimuli to ensure that they are eliciting a consistent anxious response across participants. To this end, it is also important to attempt to measure (through methods such as eye tracking) participants' attempts to regulate anxiety during the task. Often, patients report several modalities of anxiety (i.e., cognitive, emotional, bodily sensations), but there tend to be unique differences as to how each individual experiences these responses. A deeper examination of these differences could help further our understanding of how participants experience uncertainty and anticipation of negative events. We plan to continue to further this line of research with more complex and nuanced paradigms in a hope to increase our field's ability to assess and treat anxiety disorders effectively and efficiently. The neural and physiological correlates of uncertainty on responses to stimuli in anxiety disorders could be explored as biomarkers for treatment planning. Finally, the findings of this study could inform future anxiety treatment development and refinement efforts, such as targeting these regions using neurostimulation and/or neurofeedback.

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## ARTICLE INFORMATION

From the Department of Psychology (JH, BS) and the Department of Psychiatry (JH, BS, MK), University of Wisconsin-Madison, Madison, Wisconsin.

Address correspondence to Jaryd Hiser, Ph.D., at jhiser@wisc.edu.

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