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Structural evidence for involvement of a left amygdala-orbitofrontal network in subclinical anxiety

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Abstract

Functional neuroimaging implicates hyperactivity of amygdala-orbitofrontal circuitry as a common neurobiological mechanism underlying the development of anxiety. Less is known about anxiety-related structural differences in this network. In this study, a sample of healthy adults with no history of anxiety disorders completed a 3T MRI scan and self-report mood inventories. Post-processing quantitative MRI image analysis included segmentation and volume estimation of subcortical structures, which were regressed on anxiety inventory scores, with depression scores used to establish discriminant validity. We then used a quantitative vertex-based post-processing method to correlate (1) anxiety scores and (2) left amygdala volumes with cortical thickness across the whole cortical mantle. Left amygdala volumes predicted anxiety, with decreased amygdala volume associated with higher anxiety on both state and trait anxiety measures. A negative correlation between left amygdala volume and cortical thickness overlapped with a positive correlation between anxiety and cortical thickness in left lateral orbitofrontal cortex. These results suggest a structural anxiety network that corresponds with a large body of evidence from functional neuroimaging. Such findings raise the possibility that structural abnormalities may result in a greater vulnerability to anxiety or conversely that elevated anxiety symptoms may result in focal structural changes.

Keywords

Subcortical Volumes; Cortical Thickness; Fear; Morphometry; Magnetic Resonance Imaging

1. Introduction

Anxiety is characterized by a state of apprehensive expectation, fear, hyperarousal, vigilance to threat cues, avoidance behaviors, and negatively valenced cognitions (American Psychiatric Association [DSM-IV-TR], 2000). Anxiety may be transient, triggered by

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situational stressors, and attenuated by self-regulatory strategies; however, it becomes more invasive to daily functioning when fear persists without identifiable triggers or when regulation strategies fail, resulting in a more chronic anxious state. Functional neuroimaging studies identify disruption of amygdala-prefrontal circuitry as a common neurobiological mechanism underlying anxiety-related behaviors and cognitive biases (Bishop, 2007).

Converging evidence from animals and humans implicates the amygdala as the most critical structure involved in fear and anxiety (LeDoux et al., 1988; Davis, 1992; LaBar et al., 1995; LaBar and LeDoux, 1996; Phelps and LeDoux, 2005). Anxiety is the most common mental phenomenon evoked by direct electrical stimulation of the human amygdala (Halgren et al., 1978) and the amygdala is found to be hyperresponsive to anxiety-provoking stimuli (Davidson et al., 1999). Exaggerated amygdala activation is demonstrated in trait anxiety (Stein et al., 2007), post-traumatic stress disorder (PTSD) (Shin et al., 2001; Shin et al., 2004), social anxiety (Klumpp et al., 2010; Schmidt et al., 2010), and generalized anxiety disorder (GAD) (Nitschke et al., 2009), implicating this structure in subclinical anxiety and anxiety disorders.

Neurocognitive models of fear regulation propose top-down modulation of amygdala activity by prefrontal regions (Phelps and LeDoux, 2005). Activity in the ventromedial prefrontal cortex/subgenual anterior cingulate (vmPFC) is associated with retention of extinction learning (Phelps et al., 2004), as well as the ability to use cognitive emotion regulation strategies (e.g. imagining a soothing scene) to attenuate an aversive response (Delgado et al., 2008). Impaired down-regulation of anxiety is associated with the diminished integrity of amygdala-prefrontal tracts (Banks et al., 2007; Kim and Whalen, 2009; Phan et al., 2009). Likewise, the orbitofrontal cortex (OFC) is strongly implicated in anxiety expression (Milad and Rauch, 2007), most likely due to its role in both the acquisition and reversal of positive and negative stimulus-response contingencies (Schoenbaum, Chiba, and Gallagher, 2000). Damage to the orbitofrontal cortex in humans, for example, can impair the learning and reversal of stimulus-reinforcement associations, and thus the correction of behavioral responses when reinforcement contingencies change (Rolls, 2000). A dissociation within OFC is apparent with heightened medial and lateral OFC activation apparent during the processing of positive and negative reinforcement contingencies, respectively (Kringelbach and Rolls, 2004; Milad and Rauch, 2007).

Neuroanatomical models of anxiety are largely derived from functional neuroimaging studies, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) (Bishop, 2007; Sehlmeier et al., 2009; Mechias, Etkin, and Kalisch, 2010). Supportive evidence has been obtained through magnetic resonance spectroscopy (¹H-MRS) studies, which have identified anxiety-related variations in regional brain chemistry, particularly in the orbitofrontal cortex (Grachev and Apkarian, 2000a, 2000b) in healthy volunteers. Converging evidence from functional, biochemical, and structural studies provides improved ability to identify structural biomarkers in individuals at risk for anxiety disorders and to predict responsiveness to psychopharmacological intervention. However, the number of studies investigating the structural correlates of anxiety remains sparse, particularly in subclinical populations. Thickness of the vmPFC is associated with a lower skin conductance response to a conditioned stimulus during extinction retention in healthy volunteers (Milad et al., 2005; Hartley, Fischl, and Phelps, 2011). Additionally, grey matter density was found to be inversely correlated with state and trait anxiety in regions of the left amygdala, rostral anterior cingulate, posterior cingulate, and bilateral middle frontal gyrus and positively correlated with regions of the ventrolateral PFC bilaterally in healthy adults (Spampinato et al., 2009); however, these findings are limited by the use of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983) alone, which has been

demonstrated to be more strongly correlated with depression than with anxiety (Bados, Gómez-Benito, and Balaguer, 2010).

The current study investigates the relationship between self-reported anxiety symptoms and the structural anatomy of several subcortical and cortical regions in a group of healthy volunteers. We hypothesized that anxiety symptoms, as assessed by self-report on the Beck Anxiety Inventory (BAI), would be associated with decreased left amygdala volume, given prior findings of left amygdala gray matter reduction associated with anxiety (Spampinato et al., 2009). We chose to use the BAI as a primary measure of anxiety as it was developed to discriminate between anxiety and depression (Beck et al., 1988) and has been demonstrated to perform superiorly to the STAI in this regard (Creamer, Foran, and Bell, 1995; Bados et al., 2010). The Beck Depression Inventory-II (BDI-II) was used to test the discriminant validity of the relationship between anxiety and amygdala volumes. We also administered the trait scale of the STAI to a subset of our sample in order to compare our findings with a previous report that used a different structural measure (Spampinato et al., 2009). In order to investigate cortical correlates of anxiety symptoms, we used whole brain surface-based analyses, modeling the relationship between cortical thickness and BAI and STAI scores across the entire cortical mantle. To further explore structural network characteristics, we used left amygdala volumes as a correlate in a whole brain cortical thickness analysis, a method that has previously been used to test a “statistically inferred” hippocampal-cortical network (Bernhardt et al., 2008). We hypothesized that there would be a regional convergence of structural correlates between the results from this analysis and the results from analyses of whole brain cortical thickness correlations with BAI and STAI scores, particularly in ventromedial prefrontal and orbitofrontal regions.

2. Methods

2.1. Participants

A sample of 34 individuals were recruited by advertisement from the community and gave consent to participate in a full neuropsychological battery and MRI scan, with no specific mention of anxiety in the advertisement. Although a formal structured clinical interview was not administered, specific questions were asked to screen for prior history of schizophrenia, mood disorders, substance abuse/dependence, and neurological disorders in an initial interview. Health behaviors such as daily tobacco use, caffeine intake, alcohol intake, and exercise level were assessed. Participants were excluded from analyses if they reported any prior history of depression, anxiety, psychosis, substance abuse, psychotropic medication use, or neurological disorders (e.g. epilepsy, stroke, cardiovascular disease, movement disorders, brain injury, brain infection, brain tumor, dementia, or prolonged loss of oxygen). Participants were also excluded if their BAI score was greater than 26 or their BDI-II score was greater than 29; which are established cut-off scores for severe anxiety and depression (Beck and Steer, 1990; Beck, Steer, and Brown, 1996). Four participants were excluded due to report of prior mood symptoms significant enough to seek treatment. One participant was excluded from analysis due to elevated BDI and BDI-II scores. Right-handedness was determined by positive scores on the Edinburgh Handedness Inventory (Oldfield, 1971). Three individuals were excluded due to negative handedness scores indicating left-handedness. This resulted in 25 (13 males/12 females) right-handed participants, all of whom denied any history of neurological disorders. A subset of this sample (N=18; 10 males/8 females) also completed the trait subscale of the STAI. All participants were above a borderline level of IQ functioning as established through administration of Wechsler Adult Intelligence Scale – 3rd Edition (WAIS-III), a standardized measure of intellectual functioning. See Table 1 for additional sample demographics. This study was approved by the Institutional Review Board of New York University.

2.2. Mood assessment

All subjects completed the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory-II (BDI-II). The Beck Anxiety Inventory is a 21-item self-report inventory used to assess the intensity of somatic (hands trembling, face flushed, heart pounding) and cognitive (feeling terrified, fearing the worst, fear of losing control, fear of dying) anxiety symptoms, with each item having a scale value of 0-3. Due to its one-week time frame, the BAI is best considered a measure of state rather than trait anxiety (Creamer et al., 1995). A score of 0–7 is considered minimal; 8–15 indicates mild anxiety; 16–25 reflects moderate anxiety; and 26–63 is considered severe (Beck and Steer, 1990). The BAI scale has high internal consistency ($\alpha = .92$) and item-total correlations ranging from .30 to .71 (median = .60) (Beck et al., 1988). The BDI-II is a 21-item self-report inventory used to assess affective, somatic, and cognitive symptoms of depression, with each item having a scale value of 0 to 3. A score of 0–13 is considered minimal; 14–19 is considered mild depression; 20–28 indicates moderate depression symptoms; and 29–63 is associated with severe depression (Beck et al., 1996). The BDI-II has high internal consistency with a mean coefficient alpha of 0.81 across 15 nonpsychiatric samples, ranging from 0.73 to 0.92 (Beck, Steer, and Carbin, 1988). The STAI trait scale consists of 20 statements describing how people generally feel (e.g. confident, pleasant, blue, worried, tense) rated on a 4-point intensity scale (Spielberger et al., 1983). The STAI trait scale was found to have high internal consistency ($\alpha > .9$) and test-retest reliability coefficients ranging from 0.73 to 0.86 across multiple samples (Spielberger et al., 1983).

2.3. MRI scanning and image processing

Imaging was performed at the NYU Center for Brain Imaging on a 3T Siemens Allegra head-only MR scanner. Image acquisitions included a conventional 3-plane localizer and two T1-weighted volumes (TE = 3.25 ms, TR = 2530 ms, TI = 1.100 ms, flip angle = 7 deg, field of view (FOV) = 256 mm, voxel size = $1 \times 1 \times 1.33$ mm). Acquisition parameters were optimized for increased gray/white matter image contrast. The imaging protocol was identical for all subjects studied. The image files in DICOM format were transferred to a Linux workstation for morphometric analysis. The two T1-weighted images were rigid body registered to each other and reoriented into a common space, roughly similar to alignment based on the AC-PC line. Images were automatically corrected for spatial distortion due to gradient nonlinearity (Jovicich et al., 2006) and B1 field inhomogeneity (Sled, Zijdenbos, and Evans, 1998), registered, and averaged to improve signal-to-noise ratio. Images were further processed with the FreeSurfer (5.0.0) software package (<http://surfer.nmr.mgh.harvard.edu>).

2.4. Subcortical volume estimation

Semi-automated subcortical segmentation was performed by automated segmentation based on image intensity and by assigning a neuroanatomical label to each voxel based on a manually labeled training set and Bayesian prior information (Fischl et al., 2002). Labeling is performed by rigid-body alignment of the subject's brain to the probabilistic atlas, followed by non-linear morphing to the atlas. Labels are generated based on the prior probability of a given tissue class occurring at a specific atlas location, the likelihood of the image intensity given that tissue class, and the probability of the local spatial configuration of labels given the tissue class. The technique has previously been shown to be comparable in accuracy to manual labeling (Fischl et al., 2002). In addition, labels were manually inspected and, in rare cases, corrected if necessary. The amygdala was chosen as a region of interest for the present analysis. Five additional structures were selected to establish discriminant validity: hippocampus, amygdala, thalamus, caudate, putamen, pallidum, and accumbens. Volumes were calculated separately for each hemisphere. Each hemisphere-

specific subcortical volume was then corrected for total intracranial volume for use in all further correlational analyses.

2.5. Surface reconstruction and thickness measurements

The averaged volumetric MRI scan was used to construct models of each subject's cortical surface using an automated procedure that involves (1) segmentation of the white matter, (2) tessellation of the gray/white matter boundary, (3) inflation of the folded surface tessellation, and (4) automatic correction of topological defects. These steps are described in detail elsewhere (Dale, Fischl, and Sereno, 1999; Fischl, Sereno, and Dale, 1999; Fischl, Liu, and Dale, 2001). From this reconstructed surface, measures of cortical thickness were obtained by constructing an estimate of the gray/white matter boundary by classifying all white matter voxels in the MRI volume (Fischl and Dale, 2000). The white matter surface was refined in order to obtain submillimeter accuracy in delineating the gray/white matter junction. The surface was then deformed outward to locate the pial surface. Estimates of cortical thickness were made by measuring (1) the shortest distance from each point on the white matter surface to the pial surface, and (2) the shortest distance from each point on the pial surface to the white matter surface. Cortical thickness at each vertex was computed as the average of the two values. Maps were smoothed with a Gaussian kernel (10mm FWHM) across the surface and averaged across participants using a spherical averaging technique (Fischl et al., 1999), which accurately matches anatomically homologous regions across participants while minimizing metric distortions. For each hemisphere, a general linear model was used in two separate analyses: (1) to estimate the effects left amygdala volumes and (2) BAI and STAI scores on cortical thickness at each vertex along the cortical surface. Significance maps were corrected for multiple comparisons with cluster-based Monte-Carlo simulations with 10,000 permutations (Hayasaka and Nichols, 2003). Corrected significance values of thickness correlations with BAI scores and left amygdala volumes were mapped onto the inflated surface of the average brain reconstruction for visual display.

3. Results

3.1. Age, gender, BAI, BDI-II, and STAI scores

BAI scores in our sample ranged from 0 to 25 (mean = 5.04, SD = 6.11) and BDI-II scores ranged from 0 to 27 (mean = 5.92, SD = 6.11). Pearson correlations revealed no association between age and BAI ($r = -0.03$, $P = 0.9$) or BDI-II ($r = 0.14$, $P = 0.51$) scores. There was no association between coffee intake (cups per day: $r = .18$, $P = 0.43$), alcohol intake (drinks per week: $r = -0.16$, $P = 0.49$), exercise level (none, mild, moderate, vigorous: $\rho = -0.07$, $P = 0.75$), or tobacco use (yes or no: $t(21) = 0.31$, $P = .75$) and BAI scores. BAI and BDI-II scores were highly correlated with each other ($r = 0.67$, $P < 0.001$). One-way ANOVAs revealed that there were no differences between genders on BAI scores ($F(23) = 0.56$, $P = 0.46$ [females: mean = 6, SD = 7.36; males: mean = 4.15, SD = 4.81]) or on BDI-II scores: ($F(23) = 0.24$, $P = 0.63$ [females: mean = 5.17, SD = 7.64; males: mean = 6.62, SD = 7.09]).

STAI scores in our sub-sample ranged from 32 to 54 (mean = 42.17, SD = 6.3). There was no association between STAI scores and coffee intake ($r = -0.09$, $P = 0.75$), alcohol intake ($r = 0.17$, $P = 0.53$), exercise level ($\rho = -0.27$, $P = 0.29$), or age ($r = 0.07$, $P = 0.78$), or tobacco use (yes or no: $t(16) = -0.13$, $P = .90$). STAI scores were highly correlated with BAI scores ($r = 0.647$, $P = 0.004$) and BDI-II scores ($r = 0.606$, $P = 0.008$). One-way ANOVAs revealed that there were no differences between genders on STAI scores ($F(16) = 1.24$, $P = 0.28$ [females: mean = 44, SD = 6.41; males: mean = 40.7, SD = 6.413]).

Although there were no correlations between age and mood symptoms in our sample, prior research demonstrating robust inverse correlations between age and subcortical volumes (Walhovd et al., 2011), as well as age and cortical thickness (Salat et al., 2004), indicate the

need to control for age effects in symptom-structure analyses. Therefore, age was used as a covariate in all structural analyses.

3.2. Subcortical Volumes

Left and right amygdala volumes were corrected by dividing by total intracranial volume (volume of the cranial cavity, including CSF) (Buckner et al., 2004; Sanfilipo et al., 2004) and the results were regressed on BDI-II, BAI, and STAI scores, controlling for age. Left amygdala volumes significantly contributed to the variance in BAI scores ($b = -0.66$, $t(22) = -3.8$, $P = 0.001$) and STAI scores ($b = -0.66$, $t(17) = -2.87$, $P = 0.01$) but not BDI-II scores ($b = -0.34$, $t(22) = -1.57$, $P = 0.129$). The discriminant validity of the relationship between BAI and left amygdala scores was further tested by regressing all ICV corrected left and right subcortical volumes on BAI, BDI-II, and STAI scores, correcting for age. None of the other subcortical volumes contributed to the variance on any of the mood measures, indicating a selective relationship between the left amygdala and anxiety scores. Results from all regression analyses are presented in Table 2.

3.3. Cortical thickness analyses

3.3.1. BAI and STAI Scores—Two brain regions showed distinct structural differences associated with BAI scores (see Figure 1a). Higher anxiety symptoms were associated with thicker cortex, independent of age effects, in left hemisphere lateral orbitofrontal cortex, as well as a large temporo-parietal cluster spanning the posterior portion of the superior temporal sulcus and the inferior parietal region. A largely corresponding left hemisphere temporo-parietal region was positively associated with STAI scores (see Figure 2). There were no significant regions in the right hemisphere associated with BAI or STAI scores nor were there areas of decreased cortical thickness associated with either measure.

3.3.2. Left amygdala volumes—Increased left amygdala volume was associated with thicker cortex in left lateral orbitofrontal cortex, after correcting for age effects (see Figure 1b). There were no significant clusters in the right hemisphere nor where there areas where decreased amygdala volume was associated with a decrease in cortical thickness.

3.3.3. Conjunction Analysis—A negative correlation between left amygdala volume and cortical thickness overlapped with a positive correlation between BAI scores and cortical thickness in the left lateral orbitofrontal cortex (see Figure 3).

4. Discussion

Our results demonstrate that higher self-reported state and trait anxiety symptoms are associated with decreased left amygdala volume. This relationship was specific to anxiety symptoms, even though there was a strong correlation between self-reported anxiety and depression symptoms. This relationship was also specific to the left amygdala; it was not present with the right amygdala or any other subcortical structures. Demonstration of an inverse relationship between anxiety and left amygdala volume is consistent with a prior VBM study that found a similar inverse relationship between self-reported state and trait anxiety symptoms and left, but not right amygdala density (Spampinato et al., 2009). Together, these findings strengthen the proposal that greater subjective experience of anxiety is associated with smaller volume of the left amygdala.

Results from the vertex-based cortical thickness analyses of BAI and STAI scores suggest that an increase in thickness in distinct cortical regions also contributes to individual differences in anxiety symptoms. We found a relationship between higher BAI scores and increased cortical thickness in left lateral orbitofrontal (lOFC) and temporoparietal regions,

as well as an increase in left temporoparietal cortex thickness associated with higher STAI trait subscale scores. To test whether corresponding regions structurally covary with the size of the amygdala, we used amygdala volumes as a covariate in a cortical thickness analysis and found that *thicker* cortex in IOFC was associated with *decreased* amygdala volume, implying a degree of functional connectivity between the left amygdala and this region. Conjunction analysis revealed that a large portion of this region overlapped with a region that was found to be positively correlated with BAI scores. In other words, a decrease in amygdala volume was associated with an increase in cortical thickness in IOFC, the same region that was correlated with an increase in anxiety scores. Such results in the present sample provide strong morphometric evidence of an amygdala-orbitofrontal network involved in subclinical anxiety.

Our finding of a relationship between anxiety and *reduced* amygdala volume, although consistent with prior literature, is interesting considering evidence of exaggerated amygdala activity associated with trait anxiety in healthy controls (Barrett and Armony, 2009). The relationship between exaggerated amygdala activity and reduced morphology is also observed in mood disorders (see Ferrari et al., 2008 for a review of structural abnormalities in anxiety disorders). For example, in panic disorder, amygdala hyperactivation (van den Heuvel et al., 2005; Pillay et al., 2007) and greater resting glucose metabolism (Sakai et al., 2005) are observed along with reduced amygdala density (Massana et al., 2003). Excessive activity in the amygdala is also associated with reduced volume in depressed individuals (Siegle et al., 2003). Despite robust demonstration of amygdala hyperresponsivity in social anxiety and obsessive-compulsive disorder (OCD) (Shin and Liberzon, 2009), reduced amygdala and hippocampal volumes are found in adults with generalized social phobia (Irle et al., 2010) and reduced amygdala volume is associated with OCD symptom severity (Pujol et al., 2004). Reduced left amygdala volumes are also observed in children with anxiety disorders (Milham et al., 2005). These findings suggest that hyperresponsivity of the amygdala, regardless of whether anxiety level reaches a clinical threshold, is associated with reduced, rather than increased volume.

An opposite pattern emerges in neocortical regions associated with anxiety symptoms. Our finding of increased cortical thickness in OFC corresponds with healthy volunteer functional activation studies that show increased responsivity in OFC during fear conditioning (Morris and Dolan, 2004; Mechias et al., 2010) and during anxiety symptom provocation (Benkelfat et al., 1995; Servan-Schreiber et al., 1998; Javanmard et al., 1999; Eser et al., 2009). Our lack of findings in the vmPFC might be explained by our choice of measures, which capture the subjective experience of anxiety rather than the active modulation of it. A functional distinction is made between vmPFC, which often extends into medial orbitofrontal (mOFC) cortex, and more lateral regions of orbitofrontal cortex (IOFC). Activation of the former is observed in the extinction of conditioned fear responses (Milad, Vidal-Gonzalez, and Quirk, 2004; Milad et al., 2006; Quirk, Garcia, and González-Lima, 2006) and an increase in vmPFC thickness is associated with extinction learning (Milad et al., 2005). Anxious individuals require increased recruitment of mOFC to decrease negative emotions (Campbell-Sills et al., 2010), suggesting a role for mOFC in the attenuation of anxious responding (Rauch, Shin, and Phelps, 2006).

Conversely, hyperactivation of IOFC is observed during the anticipation of aversive stimuli (Nitschke et al., 2009), the retrieval of unhappy memories (Markowitsch et al., 2003), and cues signaling the absence of reward (Ursu and Carter, 2005), suggesting a role for increased IOFC activity in the anticipation of negative outcomes (Milad and Rauch, 2007). Interestingly, evidence for brain chemical alterations associated with anxiety was localized to the left OFC region in a magnetic resonance spectroscopy (¹H-MRS) study (Grachev and Apkarian, 2000b). Specifically, higher levels of N-Acetyl aspartate (NAA), gamma-

Aminobutyric acid (GABA), glutamine (Gln), glucose (Glc), and myo-inositol (Ins) were observed in high self-reported anxiety relative to low anxiety healthy volunteers. The authors interpreted this chemical increase to anxiety-related neuronal reorganization in OFC, a suggestion that is supported by our findings of anxiety-related cortical thickening in the same region.

Exaggerated OFC activity is robustly demonstrated in clinical anxiety populations. Neutral state studies demonstrate increased OFC activity at rest in panic disorder, with attenuation following successful imipramine treatment (Nordahl et al., 1990). Exaggerated OFC activity occurs in individuals with OCD, with the level of OFC hyperactivity correlated directly with the severity of anxiety symptoms (Ursu and Carter, 2009). Increased glucose metabolism is observed in OFC in individuals with OCD (Rauch et al., 2001) and abnormal glucose metabolism is related to treatment response, with decreased blood flow to OFC following antidepressant treatment (paroxetine) (Saxena et al., 1999). The magnitude of OFC activity predicts subsequent response to pharmacological and behavioral treatment in OCD, with lower right OFC metabolism associated with better response to clomipramine (Brody et al., 1998) and higher left OFC metabolism associated with better response to behavioral therapy (Swedo et al., 1989). Finally, improvement in OCD symptoms due to pharmacological or behavioral therapy results in reduced symptom-provocation increases in OFC activation (Nakao et al., 2005).

Although less robust, there is also evidence to support the supposition that anxiety-related OFC hyperactivity is associated with structural alterations in clinical populations. OFC volumes are positively related to ruminative worry in individuals with Generalized Anxiety Disorder (Mohlman et al., 2009). In a pediatric psychotropic drug-naïve OCD sample, greater OFC volume was associated with OCD symptom severity (Szeszko et al., 2008). However, decreased volume (Kang et al., 2004) and cortical thinning (Shin et al., 2007) of the left OFC is observed in non-drug-naïve adult OCD samples compared to healthy controls. This discrepancy suggests that psychotropic medication or prolonged disease duration may result in OFC gray matter reduction, factors that must be taken into account when interpreting findings from adult psychiatric populations.

Our demonstration of increased cortical thickness in the left temporo-parietal region associated with both BAI and STAI scores indicates that this region may play an auxiliary role in anxiety. This finding is supported by a particularly interesting observation that was made in the case of an individual with a left posterior temporal-parietal junction (TPJ) hematoma who exhibited decreased anxiety symptoms at 8-month follow-up testing (Grachev et al., 2002). Investigation of brain chemical changes with ¹H-MRS revealed decreased concentrations of NAA and Ins in the left orbitofrontal region of this individual, which was interpreted by the authors as a distant lesion effect given evidence of dense connectivity between TPJ and OFC (Pandya and Yeterian, 1998; Rolls, 2000). Although the temporo-parietal region receives minimal attention in anxiety studies, it is implicated in the subjective experience of somatic and visceral pain (Strigo et al., 2003; Dunckley et al., 2005) and contextual fear conditioning in humans activates an extensive cortico-hippocampal-amygdala network that includes inferior parietal regions (Alvarez et al., 2008).

Overall, there is robust evidence to suggest that hyperactivation of an amygdala-orbitofrontal network is related to increased anxiety symptoms and that damage to structures in this network or other key regions such as the temporo-parietal junction can result in reduced anxiety symptoms. In fact, one of the main surgical treatments for depression and OCD, the subcaudate tractotomy (Bridges et al., 1994; Jenike, 1998), involves disruption of amygdalofugal fibers to OFC, subgenual ACC, and thalamus. Post-operative follow-up of eight patients who recovered from depression following subcaudate tractotomy revealed an

insensitivity to negative feedback on the Iowa Gambling Task (Dalglish et al., 2004), a finding consistent with the putative role of the amygdala-OFC network in negative reinforcement learning (Ursu and Carter, 2005).

Correspondence between our structural results and prior findings from functional imaging and ¹H-MRS studies strengthen the proposal that function may alter structure macroscopically, with the potential for hypertrophy or hypotrophy with input, practice, or exposure. Our findings raise the possibility that increased anxiety-related activation of corticoid structures like the amygdala may result in diminished gray matter volume while increased activation of mesocortex and isocortex may result in increased gray matter. This relationship may be more complex in individuals with anxiety disorders, as prolonged disease duration and psychotropic medication use may be interacting factors.

Although increased activation of fear expression networks could result in subtle structural and chemical changes, which might increase the propensity to experience anxiety in the absence of identifiable triggers, it is also possible that developmental structural variations predispose individuals to experience exaggerated fear. In recombinant inbred strains of rats, strains with relatively small basolateral amygdala (BL) volumes exhibited stronger conditioned fear responses to both auditory tone and contextual stimuli, as compared to groups with larger BL (Yang et al., 2008), suggesting the possibility of a genetic contribution to the relationship between smaller amygdala volumes and increased fear proclivity. In other words, it remains unclear whether structural variations in the amygdala and orbitofrontal cortex are the result of experience dependent neuronal reorganization or whether genetically driven variations may predispose an individual to a heightened anxiety response. Future investigations should include a longitudinal component to directly address this issue.

A limitation of the current study is the lack of functional data that directly corresponds with our structural results. Future work should demonstrate areas of overlap between structural correlates and functional activation correlates in the same group of participants. An additional limitation is that we did not specifically ask participants about phobias or lifetime occurrence of panic attacks. Although all participants denied any current or past psychiatric symptoms for which they sought treatment, there is the possibility that they may have under-reported symptoms in the absence of specific prompting. Further limitations include our reliance on self-report measures as an index of anxiety with no objective measure of anxiety expression. This suggests that findings may be limited to the subjective experience of anxiety rather than its overt expression. However, our results correspond with prior report of increased left amygdala activity associated with instructed rather than experiential fear conditioning in the normal population, which involves a cognitive representation of fear (Phelps et al., 2001). This suggests that self-report measures may capture a unique aspect of anxiety, (i.e. cognitive representation of fear), that has previously been linked to the left amygdala. Symptom under-reporting or over-reporting are concerns when self-report measures are utilized; however, the absence of correlations between amygdala volumes and BDI-II scores suggest that symptom reporting biases alone cannot explain our findings.

In conclusion, the current study demonstrates a relationship between increased self-reported anxiety symptoms and decreased left amygdala volumes, as well as increased cortical thickness in the left lateral orbitofrontal and temporoparietal regions. Structural covariance between the left amygdala and orbitofrontal region suggest a left amygdala-orbitofrontal network associated with self-reported anxiety. Such results highlight regions which may be a focus of future longitudinal investigations of structural plasticity in response to life stress, anxiety disorder onset, and behavioral or psychopharmacological treatment response.

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References

- Alvarez RP, Biggs A, Chen G, Pine DS, Grillon C. Contextual fear conditioning in humans: Cortical-hippocampal and amygdala contributions. *Journal of Neuroscience*. 2008; 28(24):6211–6219. [PubMed: 18550763]
- American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th. Washington, DC: Author; 2000.
- Bados A, Gómez-Benito J, Balaguer G. The state-trait anxiety inventory, trait version: does it really measure anxiety? *Journal of Personality Assessment*. 2010; 92(6):560–567. [PubMed: 20954057]
- Banks SJ, Eddy KT, Angstadt M, Nathan PJ, Phan KL. Amygdala-frontal connectivity during emotion regulation. *Social Cognitive and Affective Neuroscience*. 2007; 2(4):303–312. [PubMed: 18985136]
- Barrett J, Armony JL. Influence of trait anxiety on brain activity during the acquisition and extinction of aversive conditioning. *Psychological Medicine*. 2009; 39(2):255–265. [PubMed: 18466667]
- Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *Journal of Consulting and Clinical Psychology*. 1988; 56(6):893–897. [PubMed: 3204199]
- Beck, A.; Steer, R. Beck Anxiety Inventory Manual. San Antonio: The Psychological Corporation Harcourt Brace Jovanovich, Inc; 1990.
- Beck, A.; Steer, R.; Brown, G. Manual for the Beck Depression Inventory-II. San Antonio: The Psychological Corporation; 1996.
- Beck AT, Steer RA, Carbin MG. Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. *Clinical Psychology Review*. 1988; 8(1):77–100.
- Benkelfat C, Bradwejn J, Meyer E, Ellenbogen M, Milot S, Gjedde A, Evans A. Functional neuroanatomy of CCK4-induced anxiety in normal healthy volunteers. *The American Journal of Psychiatry*. 1995; 152(8):1180–1184. [PubMed: 7625467]
- Bernhardt BC, Worsley KJ, Besson P, Concha L, Lerch JP, Evans AC, Bernasconi N. Mapping limbic network organization in temporal lobe epilepsy using morphometric correlations: insights on the relation between mesiotemporal connectivity and cortical atrophy. *NeuroImage*. 2008; 42(2):515–524. [PubMed: 18554926]
- Bishop SJ. Neurocognitive mechanisms of anxiety: an integrative account. *Trends in Cognitive Sciences*. 2007; 11(7):307–316. [PubMed: 17553730]
- Bridges PK, Bartlett JR, Hale AS, Poynton AM, Malizia AL, Hodgkiss AD. Psychosurgery: stereotactic subcaudate tractotomy. An indispensable treatment. *British Journal of Psychiatry*. 1994; 165(5):599–611. [PubMed: 7866675]
- Brody AL, Saxena S, Schwartz JM, Stoessel PW, Maidment K, Phelps ME, Baxter LRJ. FDG-PET predictors of response to behavioral therapy and pharmacotherapy in obsessive-compulsive disorder. *Psychiatry Research*. 1998; 84(1):1–6. [PubMed: 9870412]
- Buckner RL, Head D, Parker J, Fotenos A, Marcus D, Morris JC, Snyder AZ. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *NeuroImage*. 2004; 23:724–738. [PubMed: 15488422]
- Campbell-Sills L, Simmons A, Lovero K, Rochlin A, Paulus M, Stein M. Functioning of neural systems supporting emotion regulation in anxiety-prone individuals. *NeuroImage*. 2010.1016/j.neuroimage.2010.07.041
- Creamer M, Foran J, Bell R. The Beck Anxiety Inventory in a non-clinical sample. *Behaviour Research and Therapy*. 1995; 33(4):477–485. [PubMed: 7755538]
- Dalglish T, Yiend J, Bramham J, Tesdale JD, Ogilvie AD, Malhi G, Howard R. Neuropsychological processing associated with recovery from depression after stereotactic subcaudate tractotomy. *American Journal of Psychiatry*. 2004; 161(10):1913–1916. [PubMed: 15465992]

- Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *NeuroImage*. 1999; 9(2):179–194. [PubMed: 9931268]
- Davidson RJ, Abercrombie H, Nitschke JB, Putnam K. Regional brain function, emotion and disorders of emotion. *Current Opinion in Neurobiology*. 1999; 9(2):228–234. [PubMed: 10322186]
- Davis M. The Role of the Amygdala in Fear and Anxiety. *Annual Review of Neuroscience*. 1992; 15(1):353–375.
- Delgado M, Nearing K, Ledoux J, Phelps E. Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron*. 2008; 59(5):829–838. [PubMed: 18786365]
- Dunckley P, Wise RG, Aziz Q, Painter D, Brooks J, Tracey I, Chang L. Cortical processing of visceral and somatic stimulation: differentiating pain intensity from unpleasantness. *Neuroscience*. 2005; 133(2):533–542. [PubMed: 15896917]
- Eser D, Leicht G, Lutz J, Wenninger S, Kirsch V, Schüle C, Karch S, Baghai T, Pogarell O, Born C, Rupprecht R, Mulert C. Functional neuroanatomy of CCK-4-induced panic attacks in healthy volunteers. *Human Brain Mapping*. 2009; 30(2):511–522. [PubMed: 18095276]
- Ferrari M, Busatto G, McGuire P, Crippa J. Structural magnetic resonance imaging in anxiety disorders: An update of research findings. *Revista Brasileira de Psiquiatria*. 2008; 30(3):251–264. [PubMed: 18833427]
- Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97(20):11050–11055. [PubMed: 10984517]
- Fischl B, Liu A, Dale AM. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Transactions on Medical Imaging*. 2001; 20(1):70–80. [PubMed: 11293693]
- Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *NeuroImage*. 1999; 9(2):195–207. [PubMed: 9931269]
- Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron*. 2002; 33(3):341–355. [PubMed: 11832223]
- Grachev ID, Apkarian AV. Anxiety in healthy humans is associated with orbital frontal chemistry. *Molecular Psychiatry*. 2000a; 5(5):482–488. [PubMed: 11032381]
- Grachev ID, Apkarian AV. Chemical mapping of anxiety in the brain of healthy humans: an in vivo 1H-MRS study on the effects of sex, age, and brain region. *Human Brain Mapping*. 2000b; 11(4):261–272. [PubMed: 11144755]
- Grachev ID, Kumar R, Swarnkar A, Chang JK, Ramachandran TS. Effect of posterior temporal-parietal hematoma on orbital frontal chemistry in relation to a cognitive and anxiety state: a combined 1H-MRS and neuropsychological study of an unusual case as compared with 16 healthy subjects. *Journal of Chemical Neuroanatomy*. 2002; 23(3):223–230. [PubMed: 11861128]
- Halgren E, Walter RD, Cherlow DG, Crandall PH. Mental phenomena evoked by electrical stimulation of the human hippocampal formation and amygdala. *Brain: A Journal of Neurology*. 1978; 101(1):83–117. [PubMed: 638728]
- Hartley CA, Fischl B, Phelps EA. Brain structure correlates of individual differences in the acquisition and inhibition of conditioned fear. *Cerebral Cortex*. 2011 epub ahead of print at. 10.1093/cercor/bhq253
- Hayasaka S, Nichols T. Validating cluster size inference: random field and permutation methods. *NeuroImage*. 2003; 20(4):2343–2356. [PubMed: 14683734]
- van den Heuvel OA, Veltman DJ, Groenewegen HJ, Witter MP, Merkelbach J, Cath DC, van Balkom AJ, van Oppen P, van Dyck. Disorder-specific neuroanatomical correlates of attentional bias in obsessive-compulsive disorder, panic disorder, and hypochondriasis. *Archives of General Psychiatry*. 2005; 62(8):922–933. [PubMed: 16061770]
- Irle E, Ruhleder M, Lange C, Seidler-Brandler U, Salzer S, Dechent P, Weniger G, Leibing E, Leichsenring F. Reduced amygdalar and hippocampal size in adults with generalized social phobia. *Journal of Psychiatry and Neuroscience*. 2010; 35(2):126–131. [PubMed: 20184810]

- Javanmard M, Shlik J, Kennedy SH, Vaccarino FJ, Houle S, Bradwejn J. Neuroanatomic correlates of CCK-4-induced panic attacks in healthy humans: a comparison of two time points. *Biological Psychiatry*. 1999; 45(7):872–882. [PubMed: 10202575]
- Jenike MA. Neurosurgical treatment of obsessive-compulsive disorder. *British Journal of Psychiatry*. 1998; 35:79–90.
- Jovicich J, Czanner S, Greve D, Haley E, van der Kouwe A, Gollub R, Kennedy D, Schmitt F, Brown G, MacFall J, Fischl B, Dale A. Reliability in multi-site structural MRI studies: effects of gradient non-linearity correction on phantom and human data. *NeuroImage*. 2006; 30(2):436–443. [PubMed: 16300968]
- Kang D, Kim J, Choi J, Kim YI, Kim C, Youn T, Han MH, Chang KH, Kwon JS. Volumetric investigation of the frontal-subcortical circuitry in patients with obsessive-compulsive disorder. *The Journal of Neuropsychiatry and Clinical Neurosciences*. 2004; 16(3):342–349. [PubMed: 15377742]
- Kim MJ, Whalen PJ. The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2009; 29(37):11614–11618. [PubMed: 19759308]
- Klumpp H, Angstadt M, Nathan PJ, Phan KL. Amygdala reactivity to faces at varying intensities of threat in generalized social phobia: an event-related functional MRI study. *Psychiatry Research*. 2010; 183(2):167–169. [PubMed: 20609570]
- Kringelbach ML, Rolls ET. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Progress in Neurobiology*. 2004; 72(5):341–372. [PubMed: 15157726]
- LaBar KS, LeDoux JE. Partial disruption of fear conditioning in rats with unilateral amygdala damage: correspondence with unilateral temporal lobectomy in humans. *Behavioral Neuroscience*. 1996; 110(5):991–997. [PubMed: 8919001]
- LaBar KS, LeDoux JE, Spencer DD, Phelps EA. Impaired fear conditioning following unilateral temporal lobectomy in humans. *The Journal of Neuroscience*. 1995; 15(10):6846–6855. [PubMed: 7472442]
- LeDoux JE, Iwata J, Cicchetti P, Reis DJ. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *The Journal of Neuroscience*. 1988; 8(7):2517–2529. [PubMed: 2854842]
- Markowitsch HJ, Vandekerckhove MMP, Lanfermann H, Russ MO. Engagement of lateral and medial prefrontal areas in the ecphory of sad and happy autobiographical memories. *Cortex*. 2003; 39(4-5):643–665. [PubMed: 14584547]
- Massana G, Serra-Grabulosa JM, Salgado-Pineda P, Gastó C, Junqué C, Massana J, Mercader JM. Parahippocampal gray matter density in panic disorder: a voxel-based morphometric study. *The American Journal of Psychiatry*. 2003; 160(3):566–568. [PubMed: 12611840]
- Mechias M, Etkin A, Kalisch R. A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *NeuroImage*. 2010; 49(2):1760–1768. [PubMed: 19786103]
- Milad M, Quinn B, Pitman R, Orr S, Fischl B, Rauch S. Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(30):10706–10711. [PubMed: 16024728]
- Milad M, Vidal-Gonzalez I, Quirk G. Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behavioral Neuroscience*. 2004; 118(2):389–394. [PubMed: 15113265]
- Milad MR, Rauch SL. The role of the orbitofrontal cortex in anxiety disorders. *Annals of the New York Academy of Sciences*. 2007; 1121:546–561. [PubMed: 17698998]
- Milad MR, Rauch SL, Pitman RK, Quirk GJ. Fear extinction in rats: implications for human brain imaging and anxiety disorders. *Biological Psychology*. 2006; 73(1):61–71. [PubMed: 16476517]
- Milham M, Nugent A, Drevets W, Dickstein D, Leibenluft E, Ernst M, Charney D, Pine D. Selective reduction in amygdala volume in pediatric anxiety disorders: A voxel-based morphometry investigation. *Biological Psychiatry*. 2005; 57(9):961–966. [PubMed: 15860335]

- Mohlman J, Price RB, Eldreth DA, Chazin D, Glover DM, Kates WR. The relation of worry to prefrontal cortex volume in older adults with and without generalized anxiety disorder. *Psychiatry Research*. 2009; 173(2):121–127. [PubMed: 19559575]
- Morris JS, Dolan RJ. Dissociable amygdala and orbitofrontal responses during reversal fear conditioning. *NeuroImage*. 2004; 22(1):372–380. [PubMed: 15110029]
- Nakao T, Nakatani E, Nabeyama M, Yoshioka K, Tomita M, Nakagawa A. Duration effect on neuropsychological function and treatment response of OCD. *Seishin Shinkeigaku Zasshi = Psychiatria Et Neurologia Japonica*. 2005; 107(12):1286–1298. [PubMed: 16509426]
- Nitschke JB, Sarinopoulos I, Oathes DJ, Johnstone T, Whalen PJ, Davidson RJ, Kalin NH. Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. *The American Journal of Psychiatry*. 2009; 166(3):302–310. [PubMed: 19122007]
- Nordahl TE, Semple WE, Gross M, Mellman TA, Stein MB, Goyer P, King AC, Uhde TW, Cohen RM. Cerebral glucose metabolic differences in patients with panic disorder. *Neuropsychopharmacology*. 1990; 3(4):261–272. [PubMed: 2400544]
- Oldfield RC. The assessment and analysis of handedness: The Edinburgh Inventory. *Neuropsychologia*. 1971; 9(1):97–113. [PubMed: 5146491]
- Pandya, D.; Yeterian, E. Comparison of prefrontal architecture and connections. In: Roberts, AC.; Robbins, TW.; Weiskrantz, L., editors. *The Prefrontal Cortex: Executive and Cognitive Functions*. New York: Oxford University Press; 1998. p. 51–66.
- Phan KL, Orlichenko A, Boyd E, Angstadt M, Coccaro EF, Liberzon I, Arfanakis K. Preliminary evidence of white matter abnormality in the uncinate fasciculus in generalized social anxiety disorder. *Biological Psychiatry*. 2009; 66(7):691–694. [PubMed: 19362707]
- Phelps EA, O'Connor KJ, Gatenby JC, Gore JC, Grillon C, Davis M. Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience*. 2001; 4(4):437–441.
- Phelps E, Delgado M, Nearing K, LeDoux J. Extinction learning in humans: role of the amygdala and vmPFC. *Neuron*. 2004; 43(6):897–905. [PubMed: 15363399]
- Phelps E, LeDoux J. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*. 2005; 48(2):175–187. [PubMed: 16242399]
- Pillay SS, Rogowska J, Gruber SA, Simpson N, Yurgelun-Todd DA. Recognition of happy facial affect in panic disorder: an fMRI study. *Journal of Anxiety Disorders*. 2007; 21(3):381–393. [PubMed: 16860973]
- Pujol J, Soriano-Mas C, Alonso P, Cardoner N, Menchón JM, Deus J, Vallejo J. Mapping structural brain alterations in obsessive-compulsive disorder. *Archives of General Psychiatry*. 2004; 61(7):720–730. [PubMed: 15237084]
- Quirk GJ, Garcia R, González-Lima F. Prefrontal mechanisms in extinction of conditioned fear. *Biological Psychiatry*. 2006; 60(4):337–343. [PubMed: 16712801]
- Rauch SL, Dougherty DD, Cosgrove GR, Cassem EH, Alpert NM, Price BH, Nierenberg AA, Mayberg HS, Baer L, Jenike MA, Fischman AJ. Cerebral metabolic correlates as potential predictors of response to anterior cingulotomy for obsessive compulsive disorder. *Biological Psychiatry*. 2001; 50(9):659–667. [PubMed: 11704072]
- Rauch SL, Shin LM, Phelps EA. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research--past, present, and future. *Biological Psychiatry*. 2006; 60(4):376–382. [PubMed: 16919525]
- Rolls ET. The orbitofrontal cortex and reward. *Cerebral Cortex*. 2000; 10(3):284–294. [PubMed: 10731223]
- Sakai Y, Kumano H, Nishikawa M, Sakano Y, Kaiya H, Imabayashi E, Ohnishi T, Matsuda H, Yasuda A, Sato A, Diksic M, Kuboki T. Cerebral glucose metabolism associated with a fear network in panic disorder. *Neuroreport*. 2005; 16(9):927–931. [PubMed: 15931063]
- Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RSR, Busa E, Morris JC, Dale AM, Fischl B. Thinning of the cerebral cortex in aging. *Cerebral Cortex*. 2004; 14(7):721–730. [PubMed: 15054051]

- Sanfilipo MP, Benedict RH, Zivadinov R, Bakshi R. Correction for intracranial volume in analysis of whole brain atrophy in multiple sclerosis: the proportion vs. residual method. *Neuroimage*. 2004; 22(4):1732–1743. [PubMed: 15275929]
- Saxena S, Brody AL, Maidment KM, Dunkin JJ, Colgan M, Alborzian S, Phelps ME, Baxter LR. Localized orbitofrontal and subcortical metabolic changes and predictors of response to paroxetine treatment in obsessive-compulsive disorder. *Neuropsychopharmacology*. 1999; 21(6):683–693. [PubMed: 10633474]
- Schmidt S, Mohr A, Miltner WHR, Straube T. Task-dependent neural correlates of the processing of verbal threat-related stimuli in social phobia. *Biological Psychology*. 2010; 84(2):304–312. [PubMed: 20227458]
- Schoenbaum G, Chiba AA, Gallagher M. Changes in functional connectivity in orbitofrontal cortex and basolateral amygdala during learning and reversal training. *The Journal of Neuroscience*. 2000; 20(13):5179–5189. [PubMed: 10864975]
- Sehlmeyer C, Schöning S, Zwieterlood P, Pfleiderer B, Kircher T, Arolt V, Konrad C. Human fear conditioning and extinction in neuroimaging: a systematic review. *PLoS One*. 2009; 4(6):e5865. [PubMed: 19517024]
- Servan-Schreiber D, Perlstein WM, Cohen JD, Mintun M. Selective pharmacological activation of limbic structures in human volunteers: a positron emission tomography study. *The Journal of Neuropsychiatry and Clinical Neurosciences*. 1998; 10(2):148–159. [PubMed: 9608403]
- Shin LM, Whalen PJ, Pitman RK, Bush G, Macklin ML, Lasko NB, Orr SP, McInerney SC, Rauch SL. An fMRI study of anterior cingulate function in posttraumatic stress disorder. *Biological Psychiatry*. 2001; 50(12):932–942. [PubMed: 11750889]
- Shin L, Orr S, Carson M, Rauch S, Macklin M, Lasko N, Peters P, Metzger LJ, Dougherty DD, Cannistraro PA, Alpert NM, Fischman AJ, Pitman RK. Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. *Archives of General Psychiatry*. 2004; 61(2):168–176. [PubMed: 14757593]
- Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*. 2009; 35(1):169–191. [PubMed: 19625997]
- Shin Y, Yoo SY, Lee JK, Ha TH, Lee KJ, Lee JM, Kim IY, Kim SI, Kwon JS. Cortical thinning in obsessive compulsive disorder. *Human Brain Mapping*. 2007; 28(11):1128–1135. [PubMed: 17525985]
- Siegle GJ, Konecky RO, Thase ME, Carter CS. Relationships between amygdala volume and activity during emotional information processing tasks in depressed and never-depressed individuals: an fMRI investigation. *Annals of the New York Academy of Sciences*. 2003; 985:481–484. [PubMed: 12724182]
- Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Transactions on Medical Imaging*. 1998; 17(1):87–97. [PubMed: 9617910]
- Spampinato M, Wood J, De Simone V, Grafman J. Neural correlates of anxiety in healthy volunteers: a voxel-based morphometry study. *The Journal of Neuropsychiatry and Clinical Neurosciences*. 2009; 21(2):199–205. [PubMed: 19622691]
- Spielberger, C.; Gorsuch, R.; Lushene, R.; Vagg, P.; Jacobs, G. *Manual for the State-Trait Anxiety Inventory (STAI)*. Palo Alto, CA: Consulting Psychologists Press; 1983.
- Stein M, Simmons A, Feinstein J, Paulus M. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *The American Journal of Psychiatry*. 2007; 164(2):318–327. [PubMed: 17267796]
- Strigo IA, Duncan GH, Boivin M, Bushnell MC. Differentiation of visceral and cutaneous pain in the human brain. *Journal of Neurophysiology*. 2003; 89(6):3294–3303. [PubMed: 12611986]
- Swedo SE, Schapiro MB, Grady CL, Cheslow DL, Leonard HL, Kumar A, Friedland R, et al. Cerebral glucose metabolism in childhood-onset obsessive-compulsive disorder. *Archives of General Psychiatry*. 1989; 46(6):518–523. [PubMed: 2786402]
- Szeszko PR, Christian C, Macmaster F, Lencz T, Mirza Y, Taormina SP, Easter P, Rose M, Michalopoulou GA, Rosenberg DR. Gray matter structural alterations in psychotropic drug-naïve

- pediatric obsessive-compulsive disorder: an optimized voxel-based morphometry study. *The American Journal of Psychiatry*. 2008; 165(10):1299–1307. [PubMed: 18413702]
- Ursu S, Carter CS. Outcome representations, counterfactual comparisons and the human orbitofrontal cortex: implications for neuroimaging studies of decision-making. *Cognitive Brain Research*. 2005; 23(1):51–60. [PubMed: 15795133]
- Ursu S, Carter CS. An initial investigation of the orbitofrontal cortex hyperactivity in obsessive-compulsive disorder: exaggerated representations of anticipated aversive events? *Neuropsychologia*. 2009; 47(10):2145–2148. [PubMed: 19467363]
- Walhovd KB, Westlye LT, Amlie I, Espeseth T, Reinvang I, Raz N, Agartz I, Salat DH, Greve DN, Fischl B, Dale AM, Fjell AM. Consistent neuroanatomical age-related volume differences across multiple samples. *Neurobiology of Aging*. 2011; 32(5):916–932. [PubMed: 19570593]
- Yang RJ, Mozhui K, Karlsson R, Cameron HA, Williams RW, Holmes A. Variation in mouse basolateral amygdala volume is associated with differences in stress reactivity and fear learning. *Neuropsychopharmacology*. 2008; 33(11):2595–2604. [PubMed: 18185497]

Figure 1.

All results are statistical P maps thresholded at $P < 0.05$, cluster-corrected, and displayed on the group-averaged left hemisphere inflated surface. Scatterplot y-axis depicts mean cortical thickness of significant clusters. OFC=orbitofrontal cortex; TPJ=temporo-parietal junction

a. Areas with significant BAI and cortical thickness correlations. There were no significant right hemisphere regions and no regions that showed a negative correlation. **b.** Areas with significant left amygdala volume and cortical thickness correlation. There were no significant right hemisphere regions or regions that showed a positive correlation.

Figure 2.

Results are statistical P maps thresholded at $P < 0.05$, cluster-corrected, and displayed on the group-averaged left hemisphere inflated surface. Scatterplot y-axis depicts mean cortical thickness of significant cluster in the temporal parietal region and x-axis depicts STAI scores. There were no significant right hemisphere regions and no regions that showed a negative correlation with STAI scores.

Figure 3. Areas of overlap between (1) BAI scores correlated with cortical thickness, and (2) left amygdala volumes correlated with cortical thickness, displayed on the group-averaged inflated surface. Scatterplots depict the direction of correlation.

Table 1

Participant demographics				
	BAI (N=25; 13 males)		STAI (N=18; 10 males)	
	Range	Mean $\pm$ S.D.	Range	Mean $\pm$ S.D.
Age	21-62 years	40 $\pm$ 12	21-54 years	39 $\pm$ 11
Education	8-20 years	15 $\pm$ 3	8-20 years	15 $\pm$ 3
WAIS-III IQ	84-143	110 $\pm$ 18	84-143	112.8 $\pm$ 18

Table 2

BDI, BAI, and STAI Scores Regressed on Subcortical Volumes										
Structure	BDI Scores (N=25)			BAI Scores (N=25)			STAI Scores (N=18)			
	Beta	t(22)	p-value	Beta	t(22)	p-value	Beta	t(15)	p-value	
Left Amygdala	-0.34	-1.57	0.13	-0.42	-2.14	0.04	-0.66	-2.87	0.01	
Right Amygdala	-0.36	-1.67	0.11	-0.34	-1.55	0.14	-0.44	-1.49	0.16	
Left Thalamus	-0.24	-1.10	0.29	0.06	0.27	0.79	-0.05	-0.18	0.86	
Right Thalamus	-0.18	-0.78	0.44	0.05	0.20	0.84	-0.03	-0.12	0.91	
Left Caudate	-0.04	-0.16	0.87	0.10	0.40	0.70	-0.46	1.95	0.07	
Right Caudate	-0.09	-0.39	0.70	0.04	0.19	0.85	0.40	1.64	0.12	
Left Putamen	0.12	0.50	0.62	0.13	0.55	0.59	0.26	-0.22	0.83	
Right Putamen	0.12	0.53	0.60	0.16	0.70	0.49	-0.13	-0.46	0.65	
Left Pallidum	-0.23	-1.13	0.27	-0.06	0.28	0.78	0.16	0.63	0.54	
Right Pallidum	-0.28	-1.29	0.21	0.00	0.01	0.99	0.07	0.28	0.78	
Left Hippocampus	-0.08	0.40	0.70	0.03	0.13	0.90	0.22	0.88	0.39	
Right Hippocampus	-0.17	-0.82	0.42	-0.11	-0.50	0.62	0.00	-0.01	1.00	
Left Accumbens	-0.18	-0.74	0.47	-0.20	-0.84	0.41	-0.03	-0.09	0.93	
Right Accumbens	-0.02	-0.10	0.93	0.02	0.09	0.93	0.29	0.92	0.37	