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Research report

White matter integrity alterations in first episode, treatment-naive generalized anxiety disorder

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ABSTRACT

Background: Several neurobiological models of anxiety disorder posit a primary role for dysfunction of the amygdala and anterior cingulate cortex (ACC). This study tests the hypothesis that patients with generalized anxiety disorder (GAD) have abnormal white matter microstructure in the amygdala and ACC, as inferred from diffusion tensor imaging, compared with healthy controls.
Methods: Subjects were 16 right-handed, first-episode, treatment-naive GAD patients without comorbid disorders and 26 matched, healthy comparison controls. All subjects underwent diffusion tensor imaging and structural magnetic resonance imaging brain scanning. Fractional anisotropy (*FA*), a robust intravoxel measure of water self-diffusion, was compared between groups on a voxel-by-voxel basis. Associations between clinical ratings of symptom severity (i.e., the Hamilton Anxiety Scale and the Penn State Worry Questionnaire) and *FA* were assessed.
Results: Compared with healthy volunteers, patients demonstrated significantly higher *FA* in the right amygdala white matter and lower *FA* in the caudal ACC/mid-cingulate cortex white matter. Higher right amygdala *FA* correlated significantly with higher Hamilton Anxiety Scale scores and higher Penn State Worry Questionnaire scores.
Limitations: The sample size was modest and may contribute to false positive effects.
Conclusions: These findings provide the first evidence of an abnormality in white matter microstructure that involves the amygdala and the cingulate cortex in the pathogenesis of GAD, and are consistent with neurobiological models that posit a defect in emotion-related brain regions.

1. Introduction

Generalized anxiety disorder (GAD) is a common, chronic, and recurrent psychiatric condition that has a life-time prevalence rate of nearly 5.1% in the general population (Pary et al., 2003). GAD is characterized primarily by at least 6 months of symptom duration with prominent worrying and significant distress, as well as at least 3 of the following 6 symptoms on most days: fatigue, restlessness, poor concentration, irritability, muscle tension, and unsatisfying sleep (Pary et al., 2003). GAD patients are frequently users of primary care resources in western countries. In China, individuals with GAD (37.6%) were more likely to attempt suicide compared to those without GAD (4.2%) (Ma et al., 2009). Understanding the neural correlates of GAD may inform its diagnosis and treatments. However, GAD is under-researched compared with other anxiety disorders, despite its high prevalence and large impact on the healthcare system.

Functional neuroimaging studies have found hyperactivation in the amygdala in other well-studied anxiety disorders, including posttraumatic stress disorder (PTSD), social anxiety disorder (SAD), and specific phobia (Etkin and Wager, 2007). Recent pediatric GAD studies have shown hyperactivity in amygdala in response to negative emotional expression faces (McClure et al., 2007). Similar findings were evident in adult GAD patients (Nitschke et al., 2009; Etkin et al., 2010). Previous research also implicates the anterior cingulate cortex (ACC) in emotion

Abbreviations: GAD, generalized anxiety disorder; HAM-A, Hamilton Anxiety Scale; PSWQ, Penn State Worry Questionnaire; F, female; M, male

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regulation through effects on the amygdala, and suggests that deficits in ACC-amygdala connectivity may contribute to emotion dysregulation in patients with GAD (Etkin et al., 2010). In addition, structural neuroimaging in anxiety disorders have revealed volume alterations in the amygdala and the cingulate cortex (De Bellis et al., 2000; Milham et al., 2005; Asami et al., 2008; Hayano et al., 2009; Schienle et al., 2010; van Tol et al., 2010). Among these structural MRI studies, only two studies examined GAD patients. One study found significantly larger right and total amygdala volumes in pediatric GAD subjects (De Bellis et al., 2000). The other study found larger right centromedial amygdale gray matter volume in adult GAD patients (Etkin et al., 2009). Taken together, both functional and structural MRI studies converge to point to an abnormality in the ACC-amygdala circuitry in GAD.

Despite growing evidence for amygdala and cingulate abnormalities in GAD patients, there are only few published studies examining the integrity of whole-brain white matter (WM) in first-episode, treatment-naive adult patients with GAD. Diffusion tensor imaging (DTI), a well-established MRI method, can provide information about the microstructural integrity of white matter in vivo by measuring the magnitude and direction of water diffusion (Bandettini, 2009). Fractional anisotropy (*FA*) is the most common measure used to gauge the degree of the anisotropy. DTI has been used to examine white matter microstructure in a number of psychiatric disorders, including PTSD (Jackowski et al., 2008; Zhang et al., 2012), major depressive disorder (MDD) (Taylor et al., 2004; Alexopoulos et al., 2008), obsessive compulsive disorder (Szeszko et al., 2005), panic disorders (Han et al., 2008), generalized social anxiety disorder (Phan et al., 2009), and GAD (Hettema et al., 2012; Tromp do et al., 2012). Reduction of integrity in uncinate fasciculus which connects amygdala and frontal cortex was observed in both studies (Hettema et al., 2012; Tromp do et al., 2012), suggesting a key role of this major frontolimbic pathway in GAD.

The primary aim of this study was to characterize microstructural abnormalities in individuals with GAD using voxel-based DTI to examine alterations in fractional anisotropy (*FA*) as a means to evaluate whole brain white matter. Voxel-based analysis is a method that can assess comprehensive global brain structure changes without the restrictions imposed by the prior selection of regions of interest. It is highly reproducible, user-independent, and can potentially identify unsuspected anatomic abnormalities in the brain. We hypothesized that, relative to healthy control subjects, individuals with GAD would exhibit altered *FA* in the amygdala and the ACC. We related symptom severity to *FA* in previously identified brain regions.

We included only those patients with GAD who did not currently suffer from another mental disorder and did not take any psychiatric medication. GAD has high rates of psychiatric and medical comorbidity including MDD, panic disorder, social phobia, and PTSD. We chose only GAD patients without other psychiatric disorders to avoid the confounding effects of comorbidity. Treatment-naive GAD patients were studied, because prior work suggests secondary effects of anti-anxiety agents at chronic neurochemical concentrations can confound interpretation of findings (Mathew et al., 2008).

2. Methods

2.1. Subjects

A total of 16 GAD patients and 26 healthy controls participated in this study. The patients were recruited at the Mental Health Institute, Second Xiangya Hospital of Central South University, Changsha, China. Control subjects were recruited from the local community through advertisements. All subjects were right-handed, Han Chinese adults with at least nine years of formal education. The study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University, and written informed consent was obtained from all participants.

Psychiatric diagnoses based on DSM-IV Axis I disorders were determined through an informal clinical interview with a psychiatrist and the structured diagnostic Mini International Neuropsychiatric Interview (Sheehan et al., 1998, 2010). GAD was the primary diagnosis for all patients, in terms of both onset and severity. GAD patients were first-episode, treatment-naive.

Exclusion criteria for cases were meeting DSM-IV criteria for MDD, obsessive compulsive disorder (OCD), PTSD, SAD, specific disorder, panic disorder, or substance abuse within 6 months prior to the scanning, mental retardation, or current serious medical or neurological illness. No patient had ever received an evidence-based structured psychotherapy. No patients had any comorbid disorder.

Exclusion criteria for healthy control subjects were any history of psychiatric illness or family history of major psychiatric or neurological diseases in their first-degree relatives. All comparison subjects were free of any current or past axis I conditions or psychiatric medications.

All patients completed the Hamilton Anxiety Scale (Hamilton, 1959) and the Penn State Worry Questionnaire (Meyer et al., 1990).

2.2. MRI data acquisition

Images were acquired on a 1.5-T GE scanner (GE Signa, Milwaukee, Wisconsin, USA). A standard birdcage head coil was used, along with restraining foam pads to minimize head motion and to diminish the sounds of the scanner. Single-shot echo planar diffusion-weighted imaging with alignment of the anterior commissure-posterior commissure plane was used. The diffusion sensitizing gradients were applied along 13 non-collinear directions ($b=1000$ s/mm^2), together with an acquisition without diffusion weighting ($b=0$). A total of 30 contiguous axial slices with a 4-mm slice thickness and no gaps were acquired. The other acquisition parameters were: repetition time (TR)=12,000 ms, echo time (TE)=107 ms, acquisition matrix=128 × 128, field of view=240 × 240 mm^2.

T1-weighted images were acquired sagittally with a 3-D spoiled gradient echo (SPGR) pulse sequence with the following parameters: repetition time=12.1 ms, echo time=4.2 ms, field of view=240 × 240 mm, flip angle=15°, matrix size=256 × 256, slices=172, thickness=1.8 mm.

2.3. Image processing

Conventional images were assessed for the presence of abnormal anatomy and signal intensities by a board-certified radiologist. Data were analyzed in DtiStudio and quantified using fractional anisotropy (Jiang et al., 2006). For each subject, the *FA* was calculated by first normalizing the $b=0$ image to an EPI template in the standard Montreal Neurological Institute (MNI) space using Statistical Parameters Mapping (SPM5) (Wellcome Department of Cognitive Neurology, London, UK, London, http://www.fil.ion.ucl.ac.uk/spm/software/). Three pairs of eigenvalues (λ_1, λ_2, λ_3) and eigenvectors are obtained by diagonalization of the tensor matrix. The fractional anisotropy (*FA*) value was calculated according to the following