Research report

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

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A R T I C L E   I N F O

Article history:
Received 30 May 2012
Received in revised form 24 November 2012
Accepted 27 November 2012
Available online 8 January 2013

Keywords:
Amygdala
Anterior cingulate cortex
Generalized anxiety disorder
Diffusion tensor imaging
Magnetic resonance imaging

A B S T R A C T

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.

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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...
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 amygdala 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 neurologiacal 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 \((b=1000 \text{ s/mm}^2)\), 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 \((\text{TR})=12000 \text{ ms}, \text{echo time (TE)}=107 \text{ ms}, \text{acquisition matrix}=128 \times 128, \text{field of view}=240 \times 240 \text{ mm}^2\).

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

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 \((\lambda_1, \lambda_2, \lambda_3)\) and eigenvectors are obtained by diagonalization of the tensor matrix. The fractional anisotropy (FA) value was calculated according to the following: \(fa = 3 \sqrt{\frac{\sum_{i=1}^{3} \lambda_i^2}{3 \sum_{i=1}^{3} \lambda_i^2}}\).
The amygdala is the central processing unit of the fear circuit (Panksepp, 2005). Amygdala damage impairs fear conditioning in animals and produced an abnormally excessive interest in the fear-provoking stimuli (e.g., a snake) (Chudasama et al., 2009). Similarly, human patients with damage in the amygdala showed a specific deficit in the recognition of fear (Adolphs et al., 1994; Scott et al., 1997; Broks et al., 1998) and demonstrated an absence of overt fear manifestations in real-world settings (Kennedy et al., 2009; Feinstein et al., 2010). In healthy subjects, fMRI studies have found that increased proximity of threat progressively invokes augmented activity in amygdala (Mobbs et al., 2007, 2010). Recently, a small number of neuroimaging studies have begun to gather evidence with regard to the pathophysiology of GAD. Patients with GAD showed exaggerated and indiscriminate anticipatory activity in the amygdala, preceding both aversive and neutral pictures (Nitschke et al., 2009). GAD patients also exhibited non-specifically exaggerated amygdala responses during both congruent and incongruent trials in an emotional conflict task (Etkin et al., 2010). Two pediatric studies found striking, right amygdala responses to emotional faces (McClure et al., 2007; Scott et al., 2009), and anxiety, few studies have examined the microstructural alterations in the amygdala or any other brain region in GAD.

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It has been found that membrane thickness and diameter, and/or amount of parallel modulated by factors such as the degree of myelination, axonal role in mediating anxiety and chronic worry. Abnormal myelination may contribute to the enhanced FA in amygdala. More complex acquisition sequences and analysis of diffusion data may allow modelling of specific microstructural features (Assaf et al., 2008).

Cingulotomy has been used successfully to treat anxiety disorders (Ballenger, 1999), suggesting that the cingulate cortex plays a crucial role in these disorders. The ACC has been implicated in regulation of both cognitive and emotional processing (Bush et al., 2000). Furthermore, the ACC is densely interconnected with subcortical structures involved in producing basic emotions, especially the amygdala (Etkin et al., 2006; Ghoshgai et al., 2007). Weaker negative coupling between ACC and amygdala was found in patients with GAD (Etkin et al., 2010). One pediatric study did found a positive connectivity between ACC and amygdala in GAD patients, though this may reflect a compensatory response (McClure et al., 2007).

Fig. 2. Scatter plots show correlations between increased fractional anisotropy (FA) values in right amygdala and Hamilton Anxiety Scale (HAM-A) scores (left panel), as well as correlations between FA values in right amygdala and Penn State Worry Questionnaire (PSWQ) scores (right panel), in generalized anxiety disorder subjects.

Taken together, our DTI findings converge with prior studies showing abnormal amygdala and cingulate cortex responses in GAD, and may reflect or explain the aberrant patterns of these regions in response to threat in GAD. The findings of higher FA in amygdala and lower FA in cingulate cortex suggest aberrant fronto-amygdala structural connectivity in GAD, which may reflect a typical phenotype, such as emotion dysregulation.

We found significant FA difference only in the right, but not the left amygdala, which may be consistent with a recent finding showing that negative emotions were more often evoked by right than by left amygdala stimulations (Lanteaume et al., 2007). The valence lateralization hypothesis posits that the left hemisphere is dominant for positive emotions and the right hemisphere is dominant for negative emotions (Davidson, 1995). However, the possible lateralization for amygdala functions in emotion perception is still under debate (Wager et al., 2003). Future studies are needed to test formally for laterality-specific effects in a large sample size. In addition, the caudal ACC/mid-cingulate cortex found here is posterior to the area implicated in previous fMRI studies (Nitschke et al., 2009; Etkin et al., 2010; Paulesu et al., 2010). Subregions in the cingulate cortex may have different functions (Hong et al., 2009). It remains unknown why FA values in other parts of cingulate cortex are not found abnormal in the present study. Future research is needed to replicate our findings, paying special attention to the anatomic specificity in the cingulate cortex.

There are some limitations of this study which should be considered in interpreting our results. First, the sample size was modest and may contribute to type II error, obscuring true-positive effects. To address concerns about type II error, larger samples are needed to further assess WM pathology. However, the clinical group in the present study can be considered homogeneous, as all Axis I comorbidities were excluded in our study. Most previous studies have high comorbidity (e.g., with depression) in their samples that might have hampered the specificity of the results. The patients in the present study have a precise diagnosis of GAD, with no comorbid disorders at the time of the testing, and they were non-medicated. This homogenous sample allows us to draw specific conclusions about the GAD. Although neuroimaging research of other anxiety disorders could be used to extrapolate the brain circuits underlying GAD, the neural correlates of GAD may differ from those of other anxiety disorders (Blair et al., 2008). One interesting avenue for future studies is to further illuminate the neurobiological commonalities and differences among different anxiety disorders. Second, the observed microstructural difference might be linked to a disorder-related hyperresponsiveness of this structure. This abnormality may represent either a predisposition for GAD or a consequence of chronic worrying. However, the lack of correlations with illness duration in GAD patients perhaps suggests that the white matter changes may reflect a predisposition for GAD rather than the reverse. Moreover, amygdala abnormalities have been shown in individuals who have an increased risk of anxiety but no frank psychopathology. Greater amygdala activity was found in individuals with short allele of the serotonin transporter gene, which has been associated with increased anxiety-related behaviors (Hariri et al., 2002). Hyperresponsiveness in amygdala to novel faces was found in adults who had been categorized as “inhibited during infancy”, a risk factor for the development of anxiety disorders (Schwartz et al., 2003). An inverse correlation between anxiety measures and amygdala gray matter volume was found in healthy population (Spampinato et al., 2009). These findings are...
consistent with a risk marker hypothesis that amygdala abnormality relates to risk for anxiety (Perez-Edgar et al., 2007). Future longitudinal studies will be required to resolve this issue. Third, although the voxel based DTI allows us to investigate the entire brain in a fully automated manner, it may produce brain artifacts due to morphometry confounds and poor tissue specificity (i.e., partial volume effects) from image misregistration and smoothing (Alexander et al., 2001; Lee et al., 2009). Finally, our study does not have enough statistical power to test gender differences. Given a predominance of anxiety among women, future studies should examine them in GAD.

In conclusion, this first DTI study to compare subjects with GAD to healthy controls provides evidence of increased FA in the right amygdala and decreased FA in the caudal ACC. Given recent fMRI evidence of atypical amygdala hyperactivation in response to threatening stimuli in GAD, these FA alternations may be interpreted to suggest abnormal emotion processing and regulation. Abnormally elevated fear response and emotional dysregulation are the key syndromes of GAD. Our data points towards the candidate neurobiological underpinnings of these behavioral and emotional characteristics. This study provides insight into the neurobiology of GAD and may open up avenues for improved diagnosis and treatment.

Role of funding source
This study was supported by the National Natural Science Foundation of China (30830046, 81171286,81101004,91232714), the National 973 Program of China (2009CB918303), Program of Chinese Ministry of Education (2009016211001), and Hunan Natural Science Foundation (10JJ6034).

Conflict of interest
No conflict declared.

Acknowledgements
We thank Mr. Robert Wohlhueter who kindly helped with language editing.

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