Preliminary Evidence of White Matter Abnormality in the Uncinate Fasciculus in Generalized Social Anxiety Disorder

K. Luan Phan, Anton Orlichenko, Erin Boyd, Mike Angstadt, Emil F. Coccaro, Israel Liberzon, and Konstantinos Arfanakis

Background: Individuals with generalized social anxiety disorder (GSAD) exhibit exaggerated amygdala reactivity to aversive social stimuli. These findings could be explained by microstructural abnormalities in white matter (WM) tracts that connect the amygdala and prefrontal cortex, which is known to modulate the amygdala's response to threat. The goal of this study was to investigate brain frontal WM abnormalities using diffusion tensor imaging (DTI) in patients with social anxiety disorder.

Methods: A Turboprop DTI sequence was used to acquire diffusion tensor images in 30 patients with GSAD and 30 matched healthy control subjects. Fractional anisotropy, an index of axonal organization, within WM was quantified in individual subjects, and an automated voxel-based, whole-brain method was used to analyze group differences.

Results: Compared with healthy control subjects, patients had significantly lower fractional anisotropy localized to the right uncinate fasciculus WM near the orbitofrontal cortex. There were no areas of higher fractional anisotropy in patients than controls.

Conclusions: These findings point to an abnormality in the uncinate fasciculus, the major WM tract connecting the frontal cortex to the amygdala and other limbic temporal regions, in GSAD, which could underlie the aberrant amygdala-prefrontal interactions resulting in dysfunctional social threat processing in this illness.

Key Words: Connectivity, diffusion tensor imaging, fractional anisotropy, prefrontal, social phobia, uncinate fasciculus, white matter

Generalized social anxiety disorder (GSAD), also known as social phobia, is a common, chronic disorder that typically originates before adolescence and foretells significant functional impairment and psychiatric comorbidity (1). Individuals with GSAD exhibit exaggerated responses in the amygdala to social fear and anxiety provocation (2–5). Converging evidence from animal and human studies show that regulation of the amygdala and its reactivity to threat is mediated by intact functional interactions with discrete regions of the prefrontal cortex, specifically the orbital prefrontal cortex (OFC) (6,7), and recent work suggests that aberrant amygdala activation and social anxiety symptoms are related to ineffective prefrontal response during emotion regulation (8). Examination of white matter (WM) tracts that structurally connect the amygdala with prefrontal structures such as the orbital prefrontal cortex (OFC) (9) may elucidate the abnormal mechanisms that underlie the functional relationships between and within these brain regions.

Diffusion tensor imaging (DTI), a recently developed magnetic resonance imaging (MRI) technique, allows the opportunity to examine the integrity of WM microstructure and thus serves as an important tool for mapping anatomical connectivity in humans (10). DTI measures the directionality and coherence of water diffusion as reflected by the degree of anisotropy, which represents an estimate of axonal organization in the brain (11). To date, the use of DTI to examine WM tracts in phobia-related anxiety disorders has been limited.

The primary aim of this study was to use state-of-the-science Turboprop DTI (12) to examine alterations in fractional anisotropy (FA) with WM tracts in the prefrontal cortex, particularly the OFC, which has been shown in neuroanatomic studies to be reciprocally and densely connected to the amygdala (9), in subjects with GSAD. Given that reduced FA is associated with compromised axonal structure and/or organization (11), we hypothesized that relative to healthy control (HC) subjects, individuals with GSAD would exhibit lower FA in WM tracts that lead from and to the OFC.

Methods and Materials

Subjects

Sixty subjects (30 with GSAD and 30 age-, sex-, education-matched healthy control subjects [HC]) participated in this study. Demographic and clinical characteristics of the subjects are presented in Table 1. GSAD diagnosis was established using the Structured Clinical Interview for DSM-IV (SCID) with additional probes from the Social Phobia Interview (13) conducted by trained, master's-level clinical assessors, and the self-administered Liebowitz Social Anxiety Scale (LSAS) (14). None of the GSAD subjects had a current/recent depressive episode or alcohol/substance abuse (within 12 months of study entry) or another anxiety disorder that was more clinically salient or preceded GSAD. Eleven GSAD subjects had some form of past history (>12 months) of substance abuse or dependence, all of whom were in full remission at the time of study entry—five had past alcohol dependence (one of whom with hallucinogen abuse and another with cannabis abuse); five had past alcohol abuse (two of whom had cannabis abuse/dependence); and one had past opiate dependence. Subjects were excluded if they had a history of obsessive-compulsive disorder, posttraumatic stress disorder,

From the Department of Psychiatry (KLP, EB, MA, IL), University of Michigan, and the Mental Health Service (KLP, IL), VA Ann Arbor Healthcare System, Ann Arbor, Michigan; the Departments of Biomedical Engineering and Electrical and Computer Engineering (AO, KA), Illinois Institute of Technology; and the Department of Psychiatry (EFC), the University of Chicago, Chicago, Illinois.

Address correspondence to K. Luan Phan, M.D., Department of Psychiatry, University of Michigan Rachel Upjohn Building, Room 2751 4250 Plymouth Road, Ann Arbor, MI 48105. E-mail: luan@umich.edu.

Received Sep 17, 2008; revised Feb 5, 2009; accepted Feb 24, 2009.

Table 1. Demographic and Clinical Characteristics of Patients and Control Subjects

	GSAD (n = 30)		HC (n = 30)[a]			
	Mean	SD	Mean	SD	t	p
Age (Years)	27.20	7.80	29.90	8.13	1.31	.194
Education (Years)	15.67	1.58	15.76	1.45	.23	.822
Liebowitz Social Anxiety Scale	76.70	17.31	13.38	11.32	14.68	<.0001
Spielberger State Anxiety	41.17	9.85	26.40	5.56	6.08	<.0001
Spielberger Trait Anxiety	48.80	9.11	28.60	5.25	8.96	<.0001
Beck Depression Inventory	10.70	6.51	1.84	2.37	6.45	<.0001
	n	%	n	%	χ^2	p
Sex					1.71	.190
Male	15	50.00	10	33.33		
Female	15	50.00	20	66.67		
Race					.36	.838
Caucasian	24	80.00	23	76.67		
African American	5	16.67	5	16.67		
Asian	1	3.33	2	6.67		

GSAD, generalized social anxiety disorder; HC, healthy control subjects.
[a]Education data were missing from 5 HC subjects, and clinical symptomatology data were not available from 10 HC subjects.

bipolar disorder, psychotic disorder, mental retardation, or developmental disorders. Healthy control subjects had no history of a psychiatric disorder verified by SCID—Nonpatient Edition. None of the subjects had a history of a major medical or neurological illness. All subjects were right-handed (based on responses to inquiry about which dominant hand was used to write, make button presses on a mouse, throw a ball, etc.) and free for psychoactive medications (for at least 8 weeks) at the time of study entry, except for one GSAD subject who was taking buproprion. All subjects provided written informed consent, and the study was approved by the local university hospital institutional review board.

MRI Protocol

Subjects were scanned with Turboprop DTI (12,15) on a 3-T GE MRI scanner (General Electric, Waukesha, Wisconsin) using the following parameters: repetition time (TR) = 5000 msec, echo time (TE) = 94 msec, 8 spin-echoes per TR/blade, 5 k-space lines acquired per spin-echo (40 lines per blade), 128 samples per line, 16 k-space blades per image, field-of-view = 24 cm × 24 cm, 36 contiguous axial slices, slice thickness = 3 mm, 256 × 256 final image matrix. Diffusion-weighted images with b = 900 sec/mm^2 images were acquired in each slice for a set of 12 diffusion directions uniformly distributed in three-dimensional (3D) space; two b = 0 sec/mm^2 images were also acquired in each slice. Turboprop DTI has been shown to be relatively immune to susceptibility-related artifacts, image warping due to eddy currents, and motion-related distortions relative to other DTI techniques (12,15). High-resolution T1-weighted anatomic data were obtained using the 3D magnetization-prepared rapid acquisition gradient-echo (MP-RAGE) sequence and the following parameters: TE = 3.2 msec, TR = 8 msec, preparation/inversion time (TI) = 725 msec, flip angle 6°, field-of-view 24 × 24 cm, 124 sagittal slices, 1.5-mm slice thickness, 192 × 256 image matrix reconstructed to 256 × 256.

DTI Image Processing and Analysis

Details for image processing and analysis have been described elsewhere (16). In brief, the diffusion tensor model was fit to each voxel to create FA images for each subject. The Brain Extraction Tool of the software package FSL (Oxford Centre for functional MRI of the Brain, Oxford, United Kingdom) was applied on all b = 0 sec/mm^2 volumes to remove the skull and noise outside of the brain. The resulting binary brain masks were then applied on the FA maps. The FA images from all subjects were smoothed using Gaussian kernels with full width at half maximum (FWHM) of 9 mm and normalized to an FA template using nonlinear registration (Statistical Parametric Mapping, SPM5, Wellcome Department of Imaging and Neuroscience, London, United Kingdom). The estimated spatial transformations were then applied on the original FA maps before smoothing; in other words, we used unsmoothed FA maps to conduct the subsequent voxel-based analysis. Voxel-based group comparisons of FA values between the HC and GSAD subjects were performed using a General Linear Model covariate analysis, with age included in the model as a covariate (17). Requisite significance was set, a priori, to detect clusters exceeding volume larger than 100 mm^3 in which uncorrected group differences across a whole-brain voxelwise search exceeded a threshold of $p < .01$, as an attempt to balance Type I and II error rates and correct for spatial correlation in the FA data. We set a less conservative significance threshold because we had an a priori hypothesis about group differences in WM FA within tracts that connect the amygdala to the prefrontal cortex (specifically the OFC). However, given that this is the first DTI study in an anxiety disorder other than OCD, we report *all* group differences that surpassed this exploratory threshold to obviate bias and to generate new hypotheses about other areas of WM pathology. Moreover, this threshold is similar to other published whole-brain voxel-based analyses of FA between psychiatric and control groups in which frontal WM abnormalities were predicted (18–22). Significant cluster(s) were overlaid on averaged FA maps and diffusion anisotropy color maps, and then localized using a published MRI atlas of human WM (23). The voxel-based analysis was repeated for normalization based on FA maps smoothed with FWHM = 5 mm. The mean FA averaged across all voxels within the entire cluster(s) with significant differences in FA between patients and control subjects were estimated and extracted for each subject, to 1) show individual variability with a scatterplot and 2) calculate Cohen d effect size to guide future studies. In addition, to clarify group differences in FA, measures of diffusivity such as trace, primary, secondary, and tertiary eigenvalues were also extracted from significant cluster(s) to examine group differences.

Results

Relative to HCs, subjects with GSAD exhibited significantly lower FA nearby the OFC localized (by Talairach coordinates, x = right, y = anterior, z = superior) to right uncinate fasciculus (UF; 18, 18, −15; 383 voxels; t = 2.88, p = .003, uncorrected; Figure 1A). No other areas of reduced WM FA were identified in the GSAD group. Extracted FA values (mean ± SD) from the UF cluster are shown in Figure 1B to show individual variability (HC: .32 ± .02 vs. GSAD: .31 ± .02; t_{58} = 2.41, p = .02, Cohen's d = .63; Figure 1B). Although, the trace, secondary, and tertiary eigenvalues from the UF cluster of the GSAD group were slightly