ORIGINAL ARTICLE

Reduced Structural Connectivity of a Major Frontolimbic Pathway in Generalized Anxiety Disorder

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Context: Emotion regulation deficits figure prominently in generalized anxiety disorder (GAD) and in other anxiety and mood disorders. Research examining emotion regulation and top-down modulation has implicated reduced coupling of the amygdala with prefrontal cortex and anterior cingulate cortex, suggesting altered frontolimbic white matter connectivity in GAD.

Objectives: To investigate structural connectivity between ventral prefrontal cortex or anterior cingulate cortex areas and the amygdala in GAD and to assess associations with functional connectivity between those areas.

Design: Participants underwent diffusion-tensor imaging and functional magnetic resonance imaging.

Setting: University magnetic resonance imaging facility.

Participants: Forty-nine patients with GAD and 39 healthy volunteer control subjects, including a matched subset of 21 patients having GAD without comorbid Axis I diagnoses and 21 healthy volunteers matched for age, sex, and education.

Main Outcome Measures: The mean fractional anisotropy values in the left and right uncinate fasciculus, as measured by tract-based analysis for diffusion-tensor imaging data.

Results: Lower mean fractional anisotropy values in the bilateral uncinate fasciculus indicated reduced frontolimbic structural connectivity in patients with GAD. This reduction in uncinate fasciculus integrity was most pronounced for patients without comorbidity and was not observed in other white matter tracts. Across all participants, higher fractional anisotropy values were associated with more negative functional coupling between the pregenual anterior cingulate cortex and the amygdala during the anticipation of aversion.

Conclusions: Reduced structural connectivity of a major frontolimbic pathway suggests a neural basis for emotion regulation deficits in GAD. The functional significance of these structural differences is underscored by decreased functional connectivity between the anterior cingulate cortex and the amygdala in individuals with reduced structural integrity of the uncinate fasciculus.

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Anxiety disorders are the most common class of mental disorders, and the prevalence of generalized anxiety disorder (GAD) is as high as 5.7%.[1] A hallmark feature of GAD is excessive, uncontrollable worry. Because the emphasis of worry is on adverse events that may occur in the future, theoretical models of GAD have emphasized aberrant anticipatory processing.[2-4] A prominent model of GAD has proposed that worry serves as an implicit strategy for avoiding negative emotional experiences.[5] In this way, worry may serve as a compensatory mechanism for deficits in productive emotion regulation strategies in GAD.[5-8]

Neuroimaging research provides a promising avenue for investigating these components of GAD phenomena. In particular, more functional imaging investigations of GAD are emphasizing neural correlates of emotion regulation deficits in the disorder. Amygdala hyperactivity has been observed in some studies[4,9-12] but not in others.[13-15] Ventral regions of the prefrontal cortex (PFC), including the ventrolateral PFC and anterior cingulate cortex (ACC), have also shown abnormal patterns of activation in multiple studies.[9,11-13] These areas have been prominently featured in research on emotion regulation and top-down modulation among healthy populations,[16-24] with ven-

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tral PFC and ACC areas presumed to modulate amygdala responses to aversion and threat.[20,21,23,25-30] A recent study[9] found aberrant functional connections between ventral PFC and ACC regions and the amygdala in GAD, suggesting a neural basis for regulatory deficits in the disorder. Reduced connectivity may be associated with decreased downregulation of the amygdala, such that the elevated anxiety observed in patients with GAD may be a direct manifestation of amygdala hyperactivity. In addition, 2 recent studies[4,15] found that increased ACC activity before treatment was associated with better outcomes following an 8-week medication trial among patients with GAD, suggesting improved prognoses for patients with preserved regulatory functions of the ACC.

It is unknown whether GAD is accompanied by alterations in white matter connectivity between PFC and ACC regions and the amygdala. Reductions in the neuronal connections linking PFC and ACC regions to the amygdala may be responsible for the emotion regulation deficits and functional imaging findings aforementioned for patients with GAD. A primary candidate for testing such structural connections is the uncinate fasciculus, the major white matter tract that directly connects the amygdala to ventral regions of the PFC and ACC.[31-37] Results of recent studies using diffusion-tensor imaging (DTI) indicate a promising role for the uncinate fasciculus as a candidate marker of regulation deficits in GAD; reduced structural integrity of the uncinate fasciculus has been implicated in social anxiety disorder (SAD),[38] bipolar disorder,[39] and trait anxiety[40] and among individuals with low-expressing serotonin transporter (*5-HTTLPR* [GenBank accession number X76753]) alleles.[41] The sole prior study[42] to date on white matter in GAD did not specifically investigate the uncinate fasciculus and used a measure of diffusion different from that in the other studies.[38-41]

The primary focus of the present study was to investigate whether patients with GAD exhibited reduced structural integrity of the uncinate fasciculus (operationalized as lower fractional anisotropy [FA] values, a common measure of DTI data) in line with the research aforementioned implicating regulatory deficits and corresponding functional abnormalities in GAD.[4,6-13,15] In addition, symptom-relevant functional consequences of uncinate fasciculus structure were evaluated using functional magnetic resonance (fMR) imaging data from the same imaging session in all participants for a task that targeted anticipatory abnormalities in GAD.[2-4,43] In analyses directly comparing DTI and fMR imaging data, we used a multiple regression approach to relate individual differences in uncinate fasciculus structure to functional connectivity between the amygdala and PFC and ACC regions. We predicted that increased structural integrity of the uncinate fasciculus would be associated with more negative coupling between those regions[20,27-30,44,45] in all participants, reflecting enhanced anticipatory regulatory function in individuals with the most robust frontolimbic structural connectivity. Finally, based on prior studies[41,46-53] linking anxiety and the uncinate fasciculus to common polymorphisms affecting serotonin and brain-derived neurotrophic factor (BDNF), we also tested whether structural connectivity of the uncinate fasciculus was reduced in S/L_G carriers relative to L_A homozygotes for *5-HTTLPR*[41] and in Met carriers relative to Val homozygotes for the BDNF Val66Met polymorphism,[53] although interactions with diagnostic groups were possible.[54]

METHODS

PARTICIPANTS

Diffusion-tensor images were obtained from 88 volunteers, who were recruited through newspaper and e-mail advertisements. All participants were right-handed (based on the Edinburgh Handedness Inventory) and underwent a Structured Clinical Interview for *DSM-IV*,[55] administered by trained doctorate-level clinicians (D.J.O. and others). Forty-nine participants (30 female) were diagnosed as having GAD (**Table**). Thirteen of them had no history of other psychopathologic conditions, as determined by the Structured Clinical Interview for *DSM-IV*, while an additional 8 patients had no other current disorder (of these 8 patients, 4 were diagnosed as having past major depressive disorder [MDD], 3 with past MDD and substance abuse, and 1 with past substance abuse). The other 28 patients met criteria for a current comorbid anxiety or mood disorder; 10 of them were diagnosed as having MDD only, 5 with SAD only, 10 with MDD and SAD, and 3 with SAD and past MDD. Control subjects were 39 volunteers (19 female) with no history of psychopathologic conditions. In addition to primary analyses on the full sample, we conducted ancillary analyses on a matched sample of 21 patients (12 female) with no other current diagnosis and 21 healthy control subjects (matched for age, sex, and education).

The Table gives scores for the Hamilton Scale for Anxiety (HAM-A),[56] Hamilton Scale for Depression (HAM-D),[57] Generalized Anxiety Disorder Questionnaire,[58] and Penn State Worry Questionnaire,[59] which were administered after the Structured Clinical Interview for *DSM-IV* at the screening session. Current medication use was an exclusion criterion for this study; past medication history was collected only for the final 14 patients, of whom 6 reported no past medication use, 7 had taken antidepressant or antianxiety medications (sertraline hydrochloride, paroxetine hydrochloride, fluoxetine hydrochloride, bupropion hydrochloride, clonazepam, or alprazolam) for periods ranging from 2 months to 1 year, and 1 had tried a brief trial of a sleep medication (the drug name was unrecalled) approximately 1 year before participation. Informed consent was obtained from all participants before the experiment in accord with study approval by the institutional review board of the University of Wisconsin School of Medicine and Public Health. All individuals were paid for their participation.

DATA ACQUISITION

Diffusion-tensor images were obtained using a 3.0-T imaging system (Signa; GE Medical Systems) with a quadrature birdcage head coil. A vacuum pillow was used to minimize distortion due to head movement. Diffusion-weighted MR imaging was performed, with cardiac-gated 2-dimensional echoplanar sequence, repetition time of approximately 10 to 12 seconds (dependent on heart rate), echo time of 72 milliseconds, flip angle α of 90°, field of view of 24×24 cm, 128×128–pixel matrix (interpolated to 256×256 pixels), section thickness of 3 mm, 39 axial sections, 12 optimum noncollinear encoding directions, b value of 1000 s/mm^2 with a single image having a b value of 0 s/mm^2, and 3 excitations. Field maps for correcting geometric distortions in the DTI data were also obtained (eAp-

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Table. Demographic, Genotypic, and Symptom Information for Healthy Control Subjects and Patients With Generalized Anxiety Disorder (GAD)

Variable	Full Sample		Matched Sample	
	Control Subjects (n = 39)	Patients With GAD (n = 49)	Control Subjects (n = 21)	Patients With GAD (n = 21)
Demographics				
Age, mean (SD), y	23.85 (6.86)	27.10 (10.61)	23.14 (5.66)	24.05 (6.60)
Female sex, No. (%)	19 (48.7)	30 (61.2)	12 (57.1)	12 (57.1)
Education, mean (SD), y	16.38 (2.34)	15.73 (1.71)	16.29 (2.45)	16.24 (1.81)
Racial/ethnic background, No.				
Europe	30	39	16	14
Africa	2	3	2	2
Far East Asia	6	5	2	4
Undeclared	1	2	1	1
BDNF grouping, No. (%)				
Val/Val	24 (61.5)	27 (55.1)	13 (61.9)	10 (47.6)
Met carrier	13 (33.3)	16 (32.7)	7 (33.3)	7 (33.3)
Met/Met	4 (10.3)	2 (4.1)	1 (4.8)	0
Val/Met	9 (23.1)	14 (28.6)	6 (28.6)	7 (33.3)
Missing	2 (5.1)	6 (12.2)	1 (4.8)	4 (19.0)
5-HTTLPR grouping, No. (%)				
L_A/L_A	9 (23.1)	12 (24.5)	5 (23.8)	5 (23.8)
S/L_G carrier	28 (71.8)	33 (67.3)	15 (71.4)	15 (71.4)
S_A/S_A	7 (17.9)	8 (16.3)	6 (28.6)	5 (23.8)
S_A/L_G	1 (2.6)	2 (4.1)	0	1 (4.8)
S_A/L_A	18 (46.2)	20 (40.8)	9 (42.9)	7 (33.3)
L_G/L_A	2 (5.1)	3 (6.1)	0	2 (9.5)
Missing	2 (5.1)	4 (8.2)	1 (4.8)	1 (4.8)
Symptom measure score, mean (SD)				
HAM-A	1.64 (1.61)	18.90 (7.53)	1.52 (1.37)	17.62 (7.33)
HAM-D	2.62 (2.60)	27.73 (11.19)	2.05 (2.04)	22.86 (12.31)
GAD-Q	1.55 (1.30)	10.24 (1.98)	1.44 (1.44)	9.54 (1.61)
PSWQ	33.66 (8.30)	63.29 (9.24)	32.45 (6.94)	61.33 (9.82)

Abbreviations: BDNF, brain-derived neurotrophic factor; GAD-Q, Generalized Anxiety Disorder Questionnaire; HAM-A, Hamilton Scale for Anxiety; HAM-D, Hamilton Scale for Depression; PSWQ, Penn State Worry Questionnaire.

pendix; available at: http://www.archgenpsychiatry.com). Diffusion-tensor imaging allows for research of white matter integrity in vivo by measuring magnitude and orientation of water diffusion. Dense white matter tracts have highly anisotropic diffusion of water oriented parallel to the fiber bundle, while gray matter has predominantly isotropic water diffusion. A common diffusion measure, FA, describes the directional variance of mean diffusivity (MD) and is high in white matter regions with dense, well-myelinated, and parallel axon bundles. Another measure often used is MD, which describes the diffusion in all directions and is sensitive to the overall density of tissue membranes. Therefore, FA and MD reflect complementary aspects of tissue microstructure (coherence vs density).[60-63]

Whole-brain anatomical and functional images were acquired from all participants in the same imaging session (pulse sequences are given in the eAppendix). The functional paradigm implemented was an emotional anticipation task.[4,23,64,65] Participants viewed cues that were followed by a 2-second to 8-second jittered interstimulus interval and subsequent aversive and neutral pictures (full details are given in the eAppendix).

IMAGE ANALYSIS

Distortions in the diffusion-weighted images caused by eddy currents, magnetic field inhomogeneities, and head motion were corrected using affine coregistration and geometrically unwarping the echoplanar images using an FSL toolbox (FMRIB Software Library).[66] The FA and MD maps were calculated using available software (Diffusion Toolkit; trackvis.org).

Deterministic tractography was the primary method used for assessing whether patients had abnormalities in white matter integrity. The actual shape of the white matter fiber tracts, or large bundles of axons connecting distal brain regions, can be deduced by visualizing the water diffusion as tensors and then plotting lines through those tensors.[63] Tractography uses the principal direction of the tensor to reconstruct the white matter tracts of interest, and analyses are conducted on the identified tracts in their entirety (eg, uncinate fasciculus and corpus callosum).[67]

To estimate the fiber tracts, a line originating at a seed voxel was propagated following the tensor direction. This was accomplished using software (Camino; http://cds.ismrm.org/ismrm-2006/files/02759.pdf)[68] that applies a tensor deflection algorithm for deterministic tractography.[69] Fiber trajectories were terminated at voxels with FA values less than 0.15 or when the dot product between the previous and the current direction was less than 0.7. Another software package (TrackVis; trackvis.org) was used to visualize the identified tracts and to manually delineate the uncinate fasciculus and 3 control regions (cingulum, corpus callosum, and inferior frontooccipital fasciculus) in each participant using region-of-interest–based axonal tracking methods[70] (**Figure 1** and video available at http://www.archgenpsychiatry.com). The mean FA and MD values were exported for each of these structures and for the whole brain. One patient had missing data for the cingulum and another for the corpus callosum. The manual delineation was executed by D.P.M.T. and 2 trained students ($\kappa = 0.83$ for agreement).

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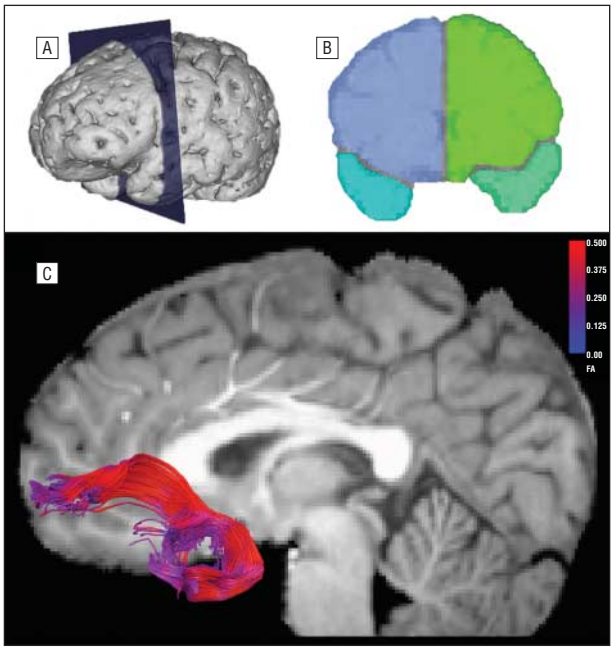

Figure 1. Region-of-interest placement for delineation of the bilateral uncinate fasciculus. A, The most posterior coronal section that showed clear separation of the frontal and temporal lobes bilaterally was identified in each individual. B, Bilateral frontal and temporal lobe seed regions of interest were then manually drawn on this section. The Boolean *AND* term was used to select only fibers that crossed through both the temporal and frontal seed regions of interest for tract-based analysis. C, Uncinate fasciculus tracts overlaid on an anatomical T1-weighted image for a single individual. FA indicates fractional anisotropy. For a 3-dimensional rendering, see the video.

In an attempt to provide converging evidence for the results obtained using the aforementioned tract-based analysis, the FA maps were coregistered to anatomical images using an optimized nonlinear registration method (DARTEL; Ashburner[71]). Voxelwise whole-brain analysis of FA maps was conducted using a computer program (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8) (eAppendix).

To assess whether differences in structural integrity of the uncinate fasciculus were associated with differences in functional connectivity, we implemented context-dependent correlation analysis, or psychophysiological interaction (PPI).[72] Psychophysiological interaction allows for the identification of brain regions in which functional coupling with a seed region is modulated by the task manipulation. Briefly, we defined the bilateral amygdala anatomically and averaged the 2 amygdalae, extracted amygdala time series data, and examined the relationship of these data with preprocessed whole-brain fMR imaging data. This analysis identified regions showing differential functional coupling with the amygdala during aversive vs neutral anticipation (eAppendix). Two individuals (1 patient and 1 control subject) were missing fMR imaging data and were excluded from the analysis.

GENETIC MATERIALS

Buccal cells were collected from participants by having them rinse with commercial mouthwash (10 mL) for 1 minute. Genotyping methods were adapted from published studies investigating variants of the serotonin transporter gene (*5-HTTLPR S* and *L* alleles; rs25531 L_A and L_G alleles)[73] and the BDNF single-nucleotide polymorphism rs6265[74] (eAppendix). Participants with *S* and L_G alleles were grouped together given evidence that these alleles are functionally equivalent.[75] In addition, because of the relative infrequency of the Met allele, analyses compared individuals having the Val/Val genotype with Met allele carriers (Val/Met and Met/Met).[53] Six individuals (4 patients and 2 control subjects) were missing data for *5-HTTLPR*, and 8 individuals (6 patients and 2 control subjects) were missing data for the BDNF Val66Met polymorphism (Table).

STATISTICAL ANALYSIS

The GAD and control groups did not differ in age ($F_{1,86}$=2.75, P=.10), sex ($F_{1,86}$=1.37, P=.25), education ($F_{1,86}$=2.28, P=.14), or whole-brain FA values ($F_{1,86}$=0.04, P=.84). Accordingly, findings were highly similar for all analyses on FA values for the uncinate fasciculus regardless of whether sex, age, education, and whole-brain FA values were included as covariates. Unless otherwise indicated, analyses included all 4 covariates to specify these sources of variance in the model rather than leaving them unspecified in the error term.[76]

All data for tract-based analyses were analyzed using commercially available software (SPSS, version 18; SPSS Inc). A group (GAD and control) × hemisphere (left and right) analysis of covariance (ANCOVA) tested group differences in the mean FA values for the left and right uncinate fasciculus. To assess the specificity of findings to FA, the following 2 additional analyses were conducted: (1) an identical ANCOVA except that MD values for the left and right uncinate fasciculus and whole-brain MD were also included as covariates and (2) an analogous ANCOVA testing group differences in the MD values. A group (GAD only, GAD comorbid, and controls) × hemisphere (left and right) ANCOVA compared patients having GAD with vs without current comorbid diagnoses. An ancillary group × hemisphere ANCOVA was conducted for the subsample of 21 patients having GAD without current comorbid diagnoses and 21 healthy controls matched for sex, age, and education. For this ANCOVA, only whole-brain FA was used as a covariate because these groupings were matched on the 3 demographic variables.

Two different analytic approaches were used for testing the specificity of findings to the uncinate fasciculus. First, ANCOVAs identical to the aforementioned primary analysis were conducted separately for the cingulum, corpus callosum, and inferior frontooccipital fasciculus. Second, for the voxelwise whole-brain DTI data, 2-sample *t* tests comparing the 2 groups were performed using a computer program (SPM8).

To directly relate DTI findings to functional connectivity data, multiple regression analyses were implemented using a computer program (AFNI, version 2; http://afni.nimh.nih.gov/afni[77]) to identify PFC and ACC regions in which functional coupling with the amygdala was correlated with uncinate fasciculus FA values. The primary analysis was for the mean uncinate fasciculus FA values; ancillary analyses were conducted for left and right uncinate fasciculus FA values separately. The dependent variable for these analyses was the standardized PPI coefficient at each voxel in the anatomically defined PFC for the contrast of aversive vs neutral anticipation. Independent variables used to predict these PPI coefficients were group, uncinate fasciculus FA values, and the group × uncinate fasciculus FA values interaction term (using the same aforementioned covariates). Analyses focused on the 2 predictors involving uncinate fasciculus FA values to identify a direct relationship between individual differences in frontolimbic structural and functional connectivity. The uncinate fasciculus FA predictor identified voxels in which FA values were related to functional coupling with the amygdala across all individuals, whereas the interaction term identified voxels in which the 2 groups differed in the relationship between FA values and functional coupling with the amygdala. Although not central to study hypotheses on the association of structural and functional con-

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nectivity, the group predictor identified voxels that showed a difference in functional coupling with the amygdala between the 2 groups (controlling for FA values). Small-volume correction for multiple comparisons using an uncorrected $P<.01$ threshold resulted in a minimum cluster size of 264 mm^3 to meet a corrected threshold of $P<.05$.

Associations of the DTI data with genetic polymorphisms (*5-HTTLPR* and BDNF Val66Met) were assessed with genotype × group × hemisphere ANCOVAs. Finally, Pearson product moment correlation coefficients were calculated within each group separately to assess associations between FA values for the uncinate fasciculus and symptom measures, including the HAM-A, HAM-D, Generalized Anxiety Disorder Questionnaire, and Penn State Worry Questionnaire. For all statistical tests, $\alpha=.05$ was used.

RESULTS

GROUP DIFFERENCES IN FRONTOLIMBIC STRUCTURAL CONNECTIVITY

For a group (GAD and control) × hemisphere (left and right) ANCOVA, a group main effect indicated that 49 patients with GAD had lower FA values in the bilateral uncinate fasciculus than 39 healthy controls ($F_{1,82}=5.773$, $P=.02$) (**Figure 2**). No other effects were significant ($P>.19$ for all). The group main effect for FA remained significant when MD values for the left and right uncinate fasciculus and whole-brain MD were also included as covariates ($F_{1,79}=6.632$, $P=.01$). For the analogous ANCOVA on MD values for the uncinate fasciculus, no effects involving group were observed ($P>.26$ for all).

To further explore the relationship between GAD and uncinate fasciculus microstructure, we conducted additional analyses on various groupings of patients with GAD in our sample. For analyses separating patients having GAD with (n=21) from those without (n=28) current comorbid diagnoses, a main effect of group ($F_{2,81}=4.065$, $P=.02$) in the absence of effects for hemisphere or group × hemisphere ($P>.78$ for all) indicated that the patients having GAD without comorbidity had lower FA values in the uncinate fasciculus than the healthy controls ($t_{57}=2.29$, $P=.01$), whereas the patients with comorbidity did not differ from either of these groups ($P>.23$ for all). Consistent with this finding, an additional analysis conducted on the matched sample of 21 patients without current comorbid Axis I disorders and 21 healthy volunteers also revealed a group main effect ($F_{1,39}=7.998$, $P=.007$) (eFigure 1) and, again, no effects of hemisphere or group × hemisphere ($P>.79$ for all). Of note, the group effect was also observed for 13 patients with no current or past comorbidity and 13 matched healthy controls ($F_{1,23}=13.36$, $P=.001$).

Tract-based analyses conducted on 3 control regions (cingulum, corpus callosum, and inferior frontooccipital fasciculus) revealed that group differences were largely specific to the uncinate fasciculus. ANCOVAs analogous to those aforementioned for the uncinate fasciculus indicated no effects involving group for any of the 3 structures in the full sample ($P>.18$ for all) or matched sample ($P>.06$ for all).

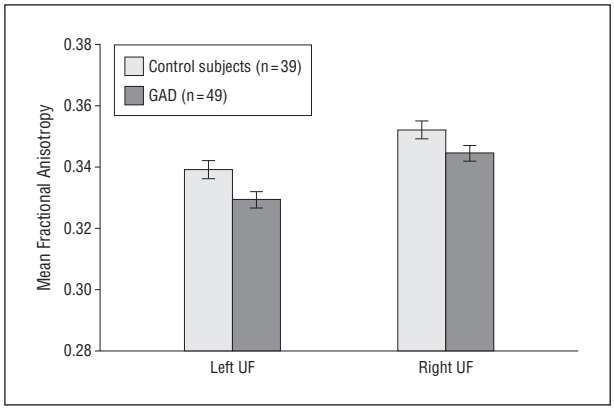

Figure 2. Patients with generalized anxiety disorder (GAD) showed reduced mean fractional anisotropy values for the uncinate fasciculus (UF) relative to healthy control subjects, as indicated by a group main effect for a group × hemisphere analysis of covariance with covariates of age, sex, education, and whole-brain fractional anisotropy values.

Voxelwise whole-brain analyses yielded confirmatory evidence of lower uncinate fasciculus FA values in patients with GAD than in controls. In the full sample, this effect was observed for the left uncinate fasciculus at uncorrected $P<.01$ and for the right at uncorrected $P<.02$ (eTable 1). The reduction in the bilateral uncinate fasciculus FA values was observed at a more stringent threshold of uncorrected $P=.005$ for the sample of 21 patients without current comorbid diagnoses and matched healthy volunteers. Consistent with the aforementioned tract-based analyses on the 3 control regions, the whole-brain analysis on the full sample indicated an absence of reliable group differences outside the uncinate fasciculus, whereas the analysis on the matched sample revealed group differences in the fornix, internal capsule, and arcuate fasciculus.

ASSOCIATIONS BETWEEN FRONTOLIMBIC STRUCTURAL CONNECTIVITY AND FUNCTIONAL CONNECTIVITY

For analyses investigating whether individual differences in uncinate fasciculus FA values were related to condition-specific functional coupling between the PFC and ACC areas and the amygdala, the pregenual ACC showed the predicted association between higher FA values and increased negative coupling with the amygdala across all participants (**Figure 3**). This effect was observed in the full sample at corrected $P<.05$ for the mean of the right and left uncinate fasciculus and for regressions of PPI coefficients on each uncinate fasciculus separately (eTable 2). An overlapping pregenual ACC cluster showed the same association with the mean uncinate fasciculus FA values for the matched sample. A left dorsolateral PFC region showed the same pattern of greater negative coupling with the amygdala for individuals with higher FA values in both the full and matched samples (eTable 2). No effects were observed for the group × uncinate fasciculus FA values interaction, indicating an absence of group differences in the relationship between FA values and functional coupling with the amygdala. Although not central to study hypotheses re-

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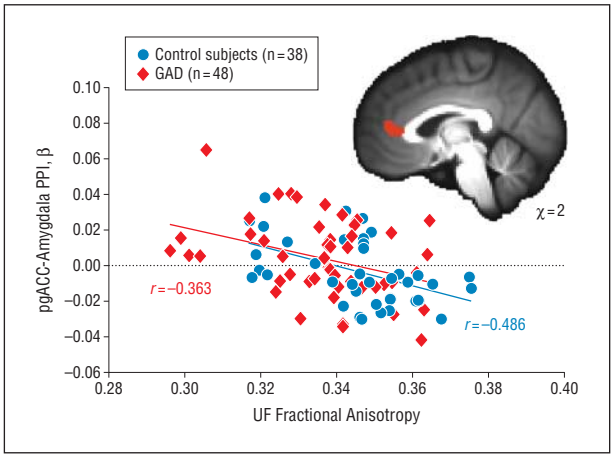

Figure 3. Participants with higher uncinate fasciculus (UF) fractional anisotropy values showed greater negative coupling between the pregenual anterior cingulate cortex (pgACC) and the amygdala during the anticipation of aversive vs neutral pictures. This relationship was seen in patients with generalized anxiety disorder (GAD [red diamonds]) and in healthy control subjects (blue circles), as evidenced by a significant pgACC cluster for the fractional anisotropy predictor but not the group or group × fractional anisotropy predictors. PPI indicates psychophysiological interaction. Note that *r* values are given for illustrative purposes only and are not intended as depicting additional statistical tests.[78,79]

lating structural and functional connectivity, group main effects were also examined (eTable 2). There was no effect of group in the pregenual ACC, but a group difference was seen in the left ventrolateral PFC, suggesting that this region's reduced functional connectivity with the amygdala in the patients was independent of group differences in uncinate fasciculus strength.

FRONTOLIMBIC STRUCTURAL CONNECTIVITY ASSOCIATIONS WITH GENOMIC AND SYMPTOM MEASURES

For *5-HTTLPR*, the genotype (S/L_G carrier and L_A/L_A) × group (GAD and control) × hemisphere (left and right) ANCOVA failed to identify effects for genotype ($F_{1,74}=0.583$, $P=.45$), group × genotype ($F_{1,74}=2.80$, $P=.10$), or any other effects involving genotype ($P>.49$ for all). Decomposition of the marginally significant group × genotype interaction indicated that among the patients S/L_G carriers had lower FA values than L_A/L_A carriers ($F_{1,39}=4.11$, $P=.049$), whereas no effect was observed among the healthy controls ($F_{1,31}=0.37$, $P=.55$). The analogous ANCOVA for the BDNF Val66Met polymorphism with genotype (Met carrier and Val/Val) failed to identify effects for genotype ($F_{1,72}=0.379$, $P=.54$), group × genotype ($F_{1,72}=0.005$, $P=.95$), or any other effects involving genotype ($P>.15$ for all). The genotype effect was also not observed for analyses conducted on each group separately ($P>.23$ for both).

Correlations calculated within each group separately revealed no reliable associations between uncinate fasciculus FA values and symptom measures. These included the HAM-A, HAM-D, Generalized Anxiety Disorder Questionnaire, and Penn State Worry Questionnaire (Bonferroni corrected $P>.16$ for all).

COMMENT

Using new tract-based analysis for DTI to assay structural connectivity among patients with GAD and healthy control subjects, we observed bilaterally reduced FA values for the uncinate fasciculus, a prominent white matter pathway connecting ventral PFC and ACC regions to the amygdala and other limbic areas. This effect was observed in the full sample of 49 patients having GAD (including those with current comorbid Axis I conditions) compared with 39 healthy controls and was particularly pronounced for patients without comorbidity. These uncinate fasciculus findings suggest a structural basis for emotion regulation deficits in GAD[6-9] and are consistent with previous functional imaging studies[4,7-13] of abnormal activation patterns in the amygdala and ACC among patients with GAD. Analyses conducted across imaging modalities elucidated the functional significance of the structural differences in the uncinate fasciculus. Across all participants, lower FA values were associated with reduced negative coupling between the ACC and amygdala, precisely the relationship expected for poorer regulatory function. Decreased structural integrity of the uncinate fasciculus in patients with GAD may have detrimental functional consequences for emotion regulation, contributing to heightened anxiety.

To our knowledge, these DTI findings represent the first report of uncinate fasciculus abnormalities in patients with GAD. Of note, a recent DTI study[42] of GAD did not present FA values for the uncinate fasciculus but instead used a method assessing the apparent diffusion coefficient, which is equivalent to MD, for circular regions of interest in each of 4 major brain lobes and the corpus callosum and found no group differences for the frontal lobe or temporal lobe. The uncinate fasciculus findings herein provide complementary support for past fMR imaging studies in GAD that have noted hyperactivity of the amygdala relative to healthy controls when study participants were involved in processes such as implicit emotion regulation and conflict monitoring,[9] anticipation of emotional (and nonemotional) images,[4] and viewing of emotional faces.[10,11] One interpretation of the amygdala hyperactivity that has frequently been observed in GAD is that patients fail to effectively recruit prefrontal circuitry that serves to regulate amygdala responses. Strong support for this hypothesis comes from work by Etkin and colleagues,[9] who demonstrated decreased coupling of the pregenual ACC and amygdala during the implicit regulation of emotional conflict in GAD. Such decreased coupling may be due to reductions in the integrity of the uncinate fasciculus, which is the primary white matter pathway connecting ventral PFC with limbic structures, including the amygdala.[31,36]

By relating DTI data for the uncinate fasciculus to fMR imaging data on a disorder-relevant task of anticipatory function, the findings herein provide evidence that reduced microstructural integrity of this pathway is likely to have functional consequences for prefrontal-limbic communication. This builds on 2 important earlier studies[39,40] that investigated relationships between DTI and fMR imaging data related to anxiety and mood disor-

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ders. Our analytic procedure reduced a multistep procedure for examining relationships among DTI, fMR imaging, and psychopathologic criteria (bipolar disorder[39] and trait anxiety[40]) to a single step that incorporates all 3 domains and allows for the simultaneous assessment of uncinate fasciculus structural integrity and diagnostic group (and their interaction) in predicting context-dependent functional connectivity with the amygdala.

Our data suggest that uncinate fasciculus integrity may be central to the previous observation in patients with GAD of reduced functional connectivity between the pregenual ACC and the amygdala.[9] Indeed, in an analysis analogous to that conducted by Etkin et al[9] with group as the sole predictor of context-dependent connectivity, patients with GAD showed reduced connectivity between the pregenual ACC and the amygdala (eFigure 2). This group main effect was not significant in our primary model that included both group and uncinate fasciculus values as predictors, reflecting the substantial overlap between individuals with GAD and those with the lowest FA values. Findings from both studies demonstrate decreased connectivity between the pregenual ACC and the amygdala in GAD, with the present study emphasizing the importance of structural contributions. In addition, our study extends previous findings[9] of altered functional connectivity to the domain of anticipatory processing. This replication across experimental paradigms provides evidence that altered pregenual ACC–amygdala circuitry may be central to pathology in GAD.

These structural and functional imaging studies point to a neurobiological basis for deficient emotion regulation abilities in individuals with GAD. Investigators examining voluntary emotion regulation frequently report activation in many regions of the PFC and ACC, which is often inversely related to amygdala activation.[25,26,28,29,43,80,81] During the anticipation of aversive images by study participants herein, we identified negative functional coupling of the pregenual ACC and the amygdala only in individuals with the highest uncinate fasciculus values. Despite the lack of explicit task instructions, it seems likely that participants nevertheless enacted preparatory regulatory strategies during the anticipation period. Our data suggest that decreased uncinate fasciculus integrity in GAD may interfere with this prefrontal regulation of amygdala activation, adding to a growing literature on altered prefrontal-amygdala communication in patients with GAD.[4,9,12] In addition to these pregenual ACC findings, individuals with higher uncinate fasciculus values showed greater negative coupling between the dorsolateral PFC and the amygdala during the anticipation of aversion. Of note, robust connections exist between the amygdala and ventral portions of the PFC and ACC,[36,82,83] while more dorsal portions of the PFC project weakly or not at all to the amygdala.[36,84,85] It may be that ventral portions of the PFC and ACC serve as critical nodes in facilitating communication between dorsal PFC regions and the amygdala during regulation of emotional responses.[29,30,86] Future research could test the hypothesis that deficient performance in patients with GAD on an explicit emotion regulation task previously shown to engage the dorsolateral PFC[25,26] is mediated by reduced integrity of the uncinate fasciculus.

Specificity of the findings was addressed in 3 ways. First, the group differences were anatomically specific to the uncinate fasciculus, as indicated by the absence of group differences elsewhere in the brain. This was determined using tract-based analyses in the cingulum, corpus callosum, and inferior frontooccipital fasciculus, as well as voxelwise whole-brain analyses.

Second, the group differences for the uncinate fasciculus were strongest for the patients having GAD without comorbidity, suggesting some degree of specificity for GAD. This observation stands in contrast to the identification of reduced uncinate fasciculus FA values in patients with SAD,[38] trait anxiety,[40] or bipolar disorder.[39] Indeed, the accumulating positive findings across different studies suggest that decreased integrity of the uncinate fasciculus may be a general risk factor for affective pathologic conditions. Future research investigating questions of comorbidity and specificity might focus in particular on uncinate fasciculus structure in unipolar depression because 23 of 28 individuals in our comorbid group had a current or past diagnosis of MDD.

Third, group differences were observed for FA but not for MD. The null findings for MD in the uncinate fasciculus are consistent with the only previously published MD findings to date for GAD.[42] Although the precise biological characteristics associated with different DTI measures are not fully known,[63] FA and MD likely quantify complementary aspects of brain microstructure. Differences in FA may reflect alterations in myelination or axonal density, whereas MD reflects the overall density of tissue membranes irrespective of fiber orientation.[63] Accordingly, findings herein for FA implicate a difference in the microstructural components that have directional dependence due to myelination or axonal density. Of note, the evidence for minimal axonal plasticity in the adult brain[87,88] is relevant to findings for the present sample, which included a broad age range. The role of uncinate fasciculus structure in the development and course of GAD and other affective disorders represents an important topic for future investigations.

Of potential relevance to the etiology of GAD, ancillary analyses examined relationships between uncinate fasciculus integrity and common genetic polymorphisms linked to anxiety. We did not replicate recent findings of reduced uncinate fasciculus FA values in healthy volunteers for the low-expressing *5-HTTLPR* allele,[41] although this pattern was observed for the patients with GAD. We also failed to replicate the finding of reduced uncinate fasciculus FA values for the BDNF Met allele.[53] Further research is needed to determine the replicability of that original finding for the BDNF Met allele[53] and to clarify whether the effects of this polymorphism on anxiety[52] and fear extinction[51,53] are mediated by the uncinate fasciculus or by a separate mechanism. A critical consideration is that, while the sample of 88 individuals is large for a neuroimaging study among patients and is greater than that for many published neuroimaging genetics studies, the sample size is insufficient for detecting the smaller effect sizes that are typical of genetic studies; therefore, these mainly negative genetic findings are inconclusive.[50]

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In summary, using DTI tract-based analysis, we identified evidence of reduced integrity of the uncinate fasciculus, a crucial white matter pathway linking ventral PFC and ACC to limbic regions, in patients with GAD. These results indicate that the altered structure of a neural pathway involved in both normative emotion regulation and fear extinction processes may contribute to atypical emotional processing in GAD. The group differences in uncinate fasciculus structural connectivity, in addition to the observed association with functional connectivity, support a model positing emotion regulation deficits in GAD[6-8] and suggest weak top-down control of amygdala reactivity. Further research is needed to determine how worry, the hallmark feature of GAD, affects the neurobiological characteristics identified herein, but its presumed function in avoiding negative emotional experiences may actually sensitize amygdala activity, resulting in a generalized state of heightened anxiety.[89] Finally, the identification of a relationship between measures of structural and functional connectivity in a circuit highly relevant for emotion regulation and anxiety disorders underscores the potential and promise for new discovery that can come about through the integration of independent modalities of imaging data.

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Online-Only Material: The eAppendix, eFigures, eTables, and video are available at http://www.archgenpsychiatry.com.
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