Compromised White Matter Tract Integrity in Schizophrenia Inferred From Diffusion Tensor Imaging

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Background: Current investigations suggest that brain white matter may be qualitatively altered in schizophrenia even in the face of normal white matter volume. Diffusion tensor imaging provides a new approach for quantifying the directional coherence and possibly connectivity of white matter fibers in vivo.

Methods: Ten men who were veterans of the US Armed Forces and met the DSM-IV criteria for schizophrenia and 10 healthy, age-matched control men were scanned using magnetic resonance diffusion tensor imaging and magnetic resonance structural imaging.

Results: Relative to controls, the patients with schizophrenia exhibited lower anisotropy in white matter, despite absence of a white matter volume deficit. In contrast to the white matter pattern, gray matter anisotropy did not distinguish the groups, even though the patients with schizophrenia had a significant gray matter volume deficit. The abnormal white matter anisotropy in patients with schizophrenia was present in both hemispheres and was widespread, extending from the frontal to occipital brain regions.

Conclusions: Despite the small sample size, diffusion tensor imaging was powerful enough to yield significant group differences, indicating widespread alteration in brain white matter integrity but not necessarily white matter volume in schizophrenia.

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There is now little controversy regarding the claim that the brains of patients with schizophrenia are structurally and functionally compromised. Abnormalities occur in both gray matter and white matter. In vivo magnetic resonance imaging studies report volume deficits more often in cortical gray than white matter1-4 and are consistent with neuropathologic observations of increased neuronal density and decreased neuropil5 presence of smaller neurons in layer III of the prefrontal cortex and absence of glial cell enlargement.6 There have also been reports of reduced prefrontal lobe white matter volume in patients with schizophrenia7,8 and of patchy signal intensity differences between patients with schizophrenia and controls that affect white matter tracts.9 Proton magnetic resonance spectroscopic imaging, which provides in vivo indices of some brain metabolites, has shown abnormally low white but not gray matter signals of N-acetyl (Nac) compounds, primarily N-acetyl aspartate, a putative marker for living mature neurons, in patients with schizophrenia who had abnormally small gray but not white matter volumes.10 The low white matter NAc signal was interpreted as potentially reflecting compromised neuronal connectivity.11-14 Evidence from postmortem studies supports the in vivo findings of anomalous white matter in schizophrenia, including selective displacement of interstitial white matter neurons in the prefrontal and temporal15 cortex and delayed myelination in frontal white matter.16,17 These neuropathologic signs may be reflected in measurements sensitive to directional coherence or connectivity of fiber tracts.

Findings of abnormal white matter integrity, together with the possibility that cortical gray matter volume deficit has a neurodevelopmental genesis, have led to the hypothesis that a cortical disconnection syndrome plays a role in the pathophysiology of schizophrenia.18-20 Perhaps the most current support for this speculation comes from diffusion tensor imaging (DTI), a relatively new magnetic resonance imaging method21,22 that can be used to quantitate the magnitude and directionality of tissue water mobility (ie, self-diffusion) in 3 dimensions.

Self-diffusion (hereafter called diffusion) is caused by random Brownian move-
SUBJECTS AND METHODS

All subjects gave written informed consent for study participation and underwent physical and psychiatric examinations. The patients were 10 men, veterans of the US Armed Forces, who met the DSM-IV criteria for schizophrenia (Table). They were 47.7±7.8 (mean ±SD) years old (range, 32-64 years) and had 13.9±1.9 years of education. Exclusion factors were DSM-IV criteria for Alcohol or Substance Abuse or Dependence within 3 months prior to scanning; posttraumatic stress disorder; significant medical illness; or head injury resulting in loss of consciousness exceeding 30 minutes. DSM-IV diagnoses were determined by consensus between a psychiatrist or clinical psychologist, who conducted a clinical interview, and a trained research assistant, who administered the Structured Clinical Interview for Diagnosis. All patients were receiving antipsychotic medications. Clinical condition was evaluated using an average of the 18-item Brief Psychiatric Rating Scale score32 (mean ±SD, 33.6±6.2) obtained by 2 raters with established reliability. Premorbid intelligence was assessed using the National Adult Reading Test33 (108.1±9.9), and parental socio-occupational status was determined using the Hollingshead 2-Factor Scale34 (2.8±1.2).

The healthy control subjects were 10 men (41.9±8.3 years; range, 30-57 years), recruited from the local community.35,36 Seven subjects were given the Structured Clinical Interview for Diagnosis and a physical examination; 3 completed a detailed questionnaire inquiring about current and past medical and psychiatric conditions, medications, and substance use.

IMAGE ACQUISITION AND PROCESSING

Anatomical Magnetic Resonance Imaging

An initial spin-echo sagittal scout image was collected (3-mm skip, 0 mm; repetition time [TR], 600 milliseconds; echo time [TE], 20 milliseconds; 256×256 pixel matrix; field of view, 24 cm). Using the midsagittal image, the line connecting the anterior and posterior commissures (AC-PC line) was identified. For tissue segmentation, a fast spin-echo (FSE) sequence was collected (TR, 2500 milliseconds; TE, 20/80 milliseconds; echo train length, 8; 5-mm skip, 0 mm; field of view, 24 cm; 256×256 pixel matrix; 18 slices beginning 2 cm below and aligned on the AC-PC line (Figure 2, A and B).

All analyses were performed blind to subject identity. Nonbrain tissue (dura, skull, and scalp) was stripped and the remaining tissue was segmented into gray matter, white matter, or cerebrospinal fluid (Figure 2, D) with a semiautomated procedure.37,38 White-matter hyperintensities segmenting as gray matter were hand-edited for inclusion in the white matter compartment.

For regional analyses, 8 images were used for volumetric quantification and manually midlined along the interhemispheric fissure to separate the hemispheres and divided according to anatomical landmarks and a priori rules into 3 lobar regions: prefrontal, temporal-parietal, and parietal-occipital (Figure 3). A prefrontal region began at the anterior margin of the slice, with the posterior extent determined by the point where the anterior cingulate and adjacent white matter met at the interhemispheric fissure. Sulcal landmarks were also considered, such that on the inferior slices the posterior border fell at the juncture between the anterior-temporal pole and the frontal cortex. On superior slices, the prefrontal region included the 3 frontal-lateral gyri. A temporal-parietal region was formed by the posterior border of the prefrontal region and the anterior border of the parietal-occipital region. Included were the temporal lobes from the level of the insula and basal ganglia to the superior extents of the lateral ventricles. A parietal-occipital region had its anterior border determined by several landmarks, including the juncture at which the cortex joins white matter along the interhemispheric fissure, posterior to lateral ventricles, and the point at which the parietal sulci are horizontal (ie, perpendicular to the

Figure 1. A computer simulation of 2-dimensional Brownian motion. Left, Paths of 10 particles starting at the same position in a condition of no spatial constraint. The particles move randomly, with a chance of moving horizontally and vertically equally in all directions (arrows), resulting in a circular displacement profile (broken circle). This movement is termed isotropic. Right, Paths of 10 particles with a physical constraint in the vertical direction (solid lines). The particles move randomly, with a greater chance of moving horizontally than vertically (arrows), resulting in an ellipsoidal displacement profile. This movement is termed anisotropic.
interhemispheric fissures). Region of interest determination was made by consensus of the 2 scorers (A.P. and E.V.S.).

**Diffusion Tensor Imaging**

A diffusion tensor data set was collected using a pulsed gradient spin-echo echo-planar imaging technique. The field of view was 24 cm; pixel matrix size, 128×128; 0 filled to 256×256 pixel matrix as required by scanner specifications; TE/TR, 106 milliseconds/6 seconds; and 18 oblique slices (5-mm skip, 0 mm) aligned with FSE slices. The duration of the diffusion gradient was 32 milliseconds, the maximum gradient strength was 1.4 G/cm, and the separation of the diffusion gradient pulses was maximized within the echo time (approximately 34 milliseconds). Gradients were always applied on 2 axes simultaneously, resulting in a total value of 860 s/mm². Diffusion was measured along 6 noncollinear directions: (Gx, Gy, Gz) = [(1,1,0), (0,1,1), (1,0,1), (−1,1,0), (0,−1,1), (1,0,−1)]. For each gradient direction, 4 diffusion-weighted images were acquired and averaged. Two additional images with no diffusion weighting were acquired and averaged. Averaging was performed after Fourier transformation. A set of cerebrospinal fluid-nulled inversion recovery images (TI < 2100 milliseconds) was acquired with no diffusion weighting as a reference that yields values between 0 (perfectly isotropic diffusion) and 1 (the hypothetical case of a cylinder infinitely long and infinitely thin). These values were plotted to produce an image (Figure 2, C) approximating the inverse of the anatomical FSE early-echo image (Figure 2, A).

According to simulation studies, rotationally invariant measures of anisotropy are “reasonably accurate and precise for signal-to-noise ratio levels greater than 20.” Our current signal-to-noise ratio of about 30 in the non–diffusion-weighted image is thus well within the range of acceptable accuracy in fractional anisotropy measurement.

**STRUCTURE/DIFFUSION ANALYSIS**

This analysis used 8 contiguous slices of the FSE and corresponding FA images that began at the anterior commissure and proceeded superiorly 40 mm. This volume encompassed the largest extent of cerebral white matter across all subjects and included the corpus callosum and central semiovale. Segmented FSE images (Figure 2, D) were registered with FA images (Figure 2, C). Fractional anisotropy voxels were characterized according to their tissue type (white matter, gray matter, and cerebrospinal fluid) and allocated to the 3 cortical regions of interest. Echo-planar warping, present in DTI, has the potential to hamper registration between DTI and FSE images; however, such effects were apparent only in the most anterior regions (Figure 2, E) and were minimized by use of tissue segmentation contours.

**STATISTICAL ANALYSIS**

The unit of DTI analysis was median FA. Group differences in FA were examined with repeated-measures analysis of variance (ANOVA) and unpaired t tests. Group differences in total FA were further tested with 3 separate analyses of covariance that used gray matter volume, white matter volume, and age as covariates. The α level for all tests was .05 (2 tailed).

### Table: Characteristics of Patients With Schizophrenia

<table>
<thead>
<tr>
<th>Subject No./Age at Scan, y</th>
<th>Education, y</th>
<th>Secondary Diagnosis</th>
<th>Past Substance Abuse/Dependence†</th>
<th>Age When Patient Last Met Diagnosis for Substance Abuse/Dependence, y‡</th>
<th>Global Assessment of Functioning Scale Score</th>
<th>Brief Psychiatric Rating Scale Score</th>
<th>Medication at Scan</th>
</tr>
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<tbody>
<tr>
<td>1/32</td>
<td>14</td>
<td>Undifferentiated</td>
<td>Cannabis abuse</td>
<td>20 (12)</td>
<td>57</td>
<td>32.0</td>
<td>Risperidone, Prolixin</td>
</tr>
<tr>
<td>2/44</td>
<td>11</td>
<td>Disorganized</td>
<td>. . .</td>
<td>. . .</td>
<td>32</td>
<td>45.0</td>
<td>Clozapine</td>
</tr>
<tr>
<td>3/46</td>
<td>13</td>
<td>Undifferentiated</td>
<td>Cannabis dependence, halucinogen abuse</td>
<td>26 (20)</td>
<td>37</td>
<td>39.0</td>
<td>Risperidone, Olanzapine</td>
</tr>
<tr>
<td>4/47</td>
<td>13</td>
<td>Undifferentiated</td>
<td>Cannabis dependence</td>
<td>20 (27)</td>
<td>52</td>
<td>32.6</td>
<td>Clozapine</td>
</tr>
<tr>
<td>5/47</td>
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<td>Undifferentiated</td>
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<td>42</td>
<td>35.0</td>
<td>Risperidone</td>
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<tr>
<td>6/48</td>
<td>16</td>
<td>Residual</td>
<td>Cannabis abuse</td>
<td>43 (5)</td>
<td>60</td>
<td>23.0</td>
<td>Clozapine</td>
</tr>
<tr>
<td>7/49</td>
<td>15</td>
<td>Residual</td>
<td>Alcohol dependence, cannabis abuse, opioid abuse</td>
<td>38 (11)</td>
<td>60</td>
<td>29.6</td>
<td>Clozapine</td>
</tr>
<tr>
<td>8/50</td>
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<td>. . .</td>
<td>. . .</td>
<td>45</td>
<td>38.6</td>
<td>Clozapine</td>
</tr>
<tr>
<td>9/50</td>
<td>11</td>
<td>Undifferentiated</td>
<td>Alcohol abuse</td>
<td>22 (32)</td>
<td>32</td>
<td>32.6</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>10/64</td>
<td>16</td>
<td>Residual</td>
<td>. . .</td>
<td>. . .</td>
<td>57</td>
<td>28.0</td>
<td>Risperidone</td>
</tr>
</tbody>
</table>

*Schizophrenia was the DSM-IV diagnosis for all subjects.
†Ellipses indicate no substance use.
‡Numbers in parentheses indicate years since patient last met this diagnosis.

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however, the so-called “diffusion tensor,” first described by Basser et al., contains information about the 3-dimensional geometry, orientation, and shape of the diffusion ellipsoid and thus fully characterizes the diffusion system. A tensor is a mathematical construct useful for describing multidimensional vector systems, and the diffusion tensor provides the apparent diffusion coefficient of a certain direction, degree of anisotropy, and primary fiber tract orientation.

Figure 2. Axial images from slice 3, 1.5 cm superior to the AC-PC line, from a single subject. A, Early echo. B, Late echo. C, Fractional anisotropy image. D, Fully processed image segmented into 3 tissue compartments (white matter=light gray, gray matter=dark gray, and cerebrospinal fluid=black). E, Fractional anisotropy image with segmentation contours overlaid to localize alignment and misalignment between anatomical and diffusion images. F, Fractional anisotropy voxels only for white matter, with segmentation contours overlaid. Note that the spatial distortion common to echo-planar imaging, especially at the frontal air-tissue boundaries (E), is confined primarily to cerebrospinal fluid and gray matter, with little distortion of white matter (F).

Figure 3. Region-of-interest boundaries, marked in black lines, are superimposed on each of the 8 fast-spin echo segmented slices used in data analysis.

Figure 4. Mean (SEM) cerebrospinal fluid, gray matter, and white matter volumes for the 10 normal control subjects and the 10 patients with schizophrenia.
The degree of anisotropy in a voxel is determined by microstructural features of the tissue in that particular voxel, such as fiber diameter and density, as well as the degree of myelination,27,28 and also by macrostructural features, such as intravoxel fiber-tract coherence.29,30 At first glance, high anisotropy may seem to be evidence of a high degree of coherence and hence highly connected tissue (such as normal white matter); however, a high degree of connectivity with low anisotropy would imply tissue with low connectivity (such as abnormal white matter); hence, a high degree of connectivity with low anisotropy can be found at the junction of merging tracts,29 where fibers with different orientations are found within the same voxel. Another example occurs in pons, where both descending and perpendicular fibers are found within a voxel. Wallerian degeneration of only the descending pathways reduces the amount of crossing fibers and increases the coherence of fibers within that particular voxel, such that the observed anisotropy increases.30 Thus, the meaning of low anisotropy must always be interpreted in the context of the anisotropy in a corresponding normal region.

Using DTI, Buchsbaum et al31 reported evidence of lower diffusion anisotropy in some inferior portions of prefrontal white matter in patients with schizophrenia (n=5) than in controls (n=6). Together with lower metabolic rates in the frontal cortex and striatum observed with positron emission tomographic scans in these same patients, these results were interpreted as diminished frontostriatal connectivity in schizophrenia.

Our controlled study of schizophrenia used DTI to quantify anisotropy determined from magnetic resonance images divided on the basis of tissue composition, hemisphere, and anatomically determined lobar regions. Our previous observations revealed gray but not white matter volume deficits, yet NAc concentration deficits occurred in white but not gray matter, suggesting compromised tissue composition in white matter.10 Therefore, we hypothesized that patients with schizophrenia would exhibit gray but not white matter volume deficits in conjunction with decreased white but not necessarily gray matter anisotropy relative to age- and sex-matched controls. Given the results of Buchsbaum et al, we anticipated that the most notable deficits would be in inferior frontal white matter in the right hemisphere.

Results

Based on the segmented FSE data, a repeated-measures ANOVA for total volume revealed a group × tissue-type interaction (F1,18=13.125, P<.002), indicating a volume deficit in gray but not white matter in the patients with schizophrenia relative to the controls (Figure 4). The FA data for gray and white matter also yielded a significant interaction (F1,18=10.521, P<.005), indicating lower FA in white but not gray matter in the patients with schizophrenia than in the controls (Figure 5). The effect size for the white matter FA group difference was 1.5 SD. The lower white matter FA persisted with 3 separate, 2-group analyses of covariance, controlling for gray matter volume, white matter volume, and age. Regional effects between groups in white matter FA were tested with a repeated-measures ANOVA across the 3 lobar regions and across hemispheres (Figure 6), with the objective of testing for a group effect (patients vs controls) and interactions involving group. This 3-way ANOVA yielded a significant effect only for group (F1,18=9.070, P<.008); none of the interactions involving group was significant.

The hypothesis that FA would be especially low in inferior frontal white matter in the right hemisphere of the schizophrenic group was tested by employing separate repeated-measures ANOVAs (2 groups × 2 hemispheres × 3 regions) for slice 2 (Figure 7, 0.5 cm superior to the AC-PC line, with inferior tips of the lateral ventricles, internal capsule, and some genu of the corpus callosum visible) and slice 3 (Figure 7, 1 cm superior to the AC-PC line, with genu and some splenium of the corpus callosum visible). For the frontal region of interest, these 2 slices appeared to correspond to those showing the greatest effects in the Buchsbaum et al31 study. The ANOVAs yielded the same results for each slice, with significant effects only for group [slice 2: F1,18 =9.843,
We compared anisotropy and volumes of equivalent regions of white and gray matter in patients with schizophrenia and controls and observed the following double dissociation. Although patients with schizophrenia and controls had an equivalent volume of white matter, this white matter exhibited lower anisotropy among patients with schizophrenia. By contrast, gray matter anisotropy did not distinguish the groups even though the schizophrenic group had a significant gray matter volume deficit. Furthermore, the abnormal white matter anisotropy in the patients with schizophrenia was present in both hemispheres and was widespread, extending from the frontal to occipital brain regions. A similar double dissociation was observed in our previous comparison of tissue volume and tissue composition of the brain metabolite NAc; patients with schizophrenia had decreased gray matter but not white matter volume and decreased white matter but not gray matter NAc concentration.

There is no convention for a DTI outcome, and the appropriateness of traditional statistical tests in the analysis of DTI data has yet to be decided. The information did not distinguish the groups even though the schizophrenic group had a significant gray matter volume deficit. Furthermore, the abnormal white matter anisotropy in the patients with schizophrenia was present in both hemispheres and was widespread, extending from the frontal to occipital brain regions. A similar double dissociation was observed in our previous comparison of tissue volume and tissue composition of the brain metabolite NAc; patients with schizophrenia had decreased gray matter but not white matter volume and decreased white matter but not gray matter NAc concentration.

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analyzed slices from the 10 normal control subjects and the 10 patients with schizophrenia.

sensitive than RA.

approximately 0.3 to 0.4) is in the range where FA is more sen-
sitivity. This range is independent of the degree of anisotropy; the an-
sor to the isotropic component; as one goes up, the other
goes down, rendering RA more sensitive to variations for
large degrees of anisotropy but not as sensitive in the mid-
range. Fractional anisotropy, on the other hand, is the
ratio of the anisotropic component to the entire tensor,
which is independent of the degree of anisotropy; the an-
isotropy that we observed in white matter (approxi-
mation). Fractional anisotropy, on the other hand, is the

The possibility of compromised white matter connec-
tivity suggested by our study must be considered
within the context of cortical gray matter pathology noted
in schizophrenia. The gray matter volume deficit is es-
pecially prominent in the heteromodal cortex,43,44 which
has extensive corticocortical and subcorticocortical in-
terconnections.32 Disruption of these interconnections
could arise from several sources: abnormally small size
or number of neurons producing commensurately fewer
than normal arbor, disturbances in the white matter struc-
ture arising from displaced interstitial neurons, or aber-
rant myelination. These white matter anomalies could
disrupt fiber coherence or might even result in compro-
mised connections. Considering neuropathologic re-
ports, either or both of these possibilities could occur in
schizophrenia. White matter growth and restructuring
occur from late prenatal development through late ado-
lescence. The cortical targets—prefrontal, superior tem-
poral, and parietal—in the heteromodal system puta-
tively disrupted in schizophrenia are critical to higher-
order cognitive functions of problem solving, working
memory, sequencing, language, and spatial orientation,
many of which are commonly impaired in patients with
schizophrenia.45,46 Our results note a potential substrate
for functional disconnection18,20 or for less dramatic dis-
ruption in brain structural organization in the form of
decreased fiber coherence. Decreased anisotropy could,
therefore, arise from underdevelopment of these oth-
erwise highly integrated neural networks, possibly from
incomplete organization of target sites during develop-
ment, and could result in decreased directional coherence
of white matter fibers or vulnerability from neurotoxic
sources, including neuroleptic exposure or symptom
exacerbation.45

Buchsbaum et al31 reported lower RA in frontal white
matter, including the anterior limb of the external cap-
sule only in the right hemisphere. Our study observed
abnormalities in these frontal regions in both hemi-
spheres as well as in nonfrontal regions. These 2 studies
differed in the analysis approach and the anisotropy mea-
sure: Buchsbaum et al used RA, whereas we used FA. They
also employed a spatial normalization method to stan-
dardized coordinates,40 which involved stretching each
cube of each subject to a common size, followed by pixel-
by-pixel t tests corrected for multiple observations. We
analyzed large regions of interest that were inherently less
anatomically specific but more robust to noise and sta-
tistically more conservative. Nonetheless, the results of
both studies converge on the possibility of compromised
frontostriatal connectivity or directional coher-
ence of white matter fibers in schizophrenia, although
our study also points to abnormalities in other regions.

Figure 8. White matter fractional anisotropy (mean and SEM) in the 3 regions of interest (frontal, temporal-parietal, and parietal-occipital) for each of the 8 analyzed slices from the 10 normal control subjects and the 10 patients with schizophrenia.
precluding perfect registration of the 2 image types. In our study, however, the misregistration appeared to be minimal except for the most anterior regions (Figure 2, E). Consequently, we used tissue segmentation as a basis for defining anatomical borders for regional FA measurements (Figure 2, F). A preferred approach may involve unwarping the DTI images, which requires the acquisition of a magnetic field map at the time of data acquisition; however, such data were not available for our analysis. Despite the limitations of our study, including its restrictions in sample size and sex, the method was powerful enough to yield group differences with a 1.5-SD effect size, indicating widespread alteration in brain white matter integrity but not necessarily white matter volume in schizophrenia.

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