Cingulate Fasciculus Integrity Disruption in Schizophrenia: A Magnetic Resonance Diffusion Tensor Imaging Study

Marek Kubicki, Carl-Fredrik Westin, Paul G. Nestor, Cynthia G. Wible, Melissa Frumin, Stephan E. Maier, Ron Kikinis, Ferenc A. Jolesz, Robert W. McCarley, and Martha E. Shenton

Background: *Evidence suggests that a disruption in limbic system network integrity and, in particular, the cingulate gyrus (CG), may play a role in the pathophysiology of schizophrenia; however, the cingulum bundle (CB), the white matter tract furnishing both input and output to CG, and the most prominent white matter fiber tract in the limbic system, has not been evaluated in schizophrenia using the new technology of diffusion tensor imaging (DTI).*

Methods: *We used line scan DTI to evaluate diffusion in the CB in 16 male schizophrenia patients and 18 male control subjects, group-matched for age, parental socioeconomic status, and handedness. We acquired 4-mm-thick coronal slices through the entire brain. Maps of fractional anisotropy (FA) were generated to quantify diffusion within the left and right CB on eight slices that included the central portion of the CB.*

Results: *Results showed group differences, bilaterally, in area and mean FA for CB, where patients showed smaller area and less anisotropy than controls. For patients, decreased left CB correlated significantly with attention and working memory measures as assessed by the Wisconsin Card Sorting Test.*

Conclusions: *These data provide strong evidence for CB disruptions in schizophrenia, which may be related to disease-related attention and working memory abnormalities.* Biol Psychiatry 2003;54:1171–1180 © *2003 Society of Biological Psychiatry*

Key Words: Cingulate fasciculus, diffusion, MRI, diffusion tensor imaging, anisotropy, executive function

From the Clinical Neuroscience Division, Laboratory of Neuroscience, Boston VA Healthcare System—Brockton Division, and Department of Psychiatry, Harvard Medical School (MK, PGN, CGW, MF, RWM, MES), Brockton, Massachusetts, and Surgical Planning Laboratory, MRI Division, Department of Radiology, Brigham and Women's Hospital, Harvard Medical School (MK, C-FW, CGW, SEM, RK, FAJ, MES), Boston, Massachusetts.

Address reprint requests to Drs. Martha E. Shenton and Robert W. McCarley, Department of Psychiatry-116A, VA Boston Health Care System-Brockton Division, Harvard Medical School, 940 Belmont Street, Brockton, MA 02301.

Received December 23, 2002; revised April 1, 2003; accepted April 2, 2003.

Introduction

The cingulum bundle (CB) is the white matter tract that underlies, and provides, the cingulate cortex with connections to and from other components of Broca's "grand limbic lobe." Of note, cingulate gyrus abnormalities have been reported in morphometric studies of schizophrenia and include bilateral decrease of cingulate gyrus gray matter volume and decreased gray matter density in voxel-based morphometric studies (Crespo-Facorro et al 2000; Goldstein et al 1999; Sigmudsson et al 2001). Moreover, histopathologic studies of schizophrenia have provided evidence for decreased cortical thickness, decreased pyramidal neuron size, and a decrease in density in both pyramidal and nonpyramidal neurons as well as deficits in small interneurons within the cingulate gyrus (Benes et al 1992, 2001; Bouras et al 2001).

The anatomy and the connections of the cingulate gyrus have been described in detail (Goldman-Rakic 1988; Pandya and Seltzer 1982; Vogt et al 1979). In cytoarchitectonics, connectivity, and function, there appear to be distinct differences between the anterior and posterior portions. The anterior–agranular, motor-related cortex is strongly interconnected with amygdala, nucleus accumbens, medial dorsal thalamus, and dorsolateral prefrontal cortex, whereas the posterior–granular, sensory-related cortex is interconnected with temporal association cortex, medial temporal cortex, and parietal and orbitofrontal cortex. The anterior division is, in fact, implicated more often in schizophrenia-related dysfunction than is the posterior division, and neuroimaging data suggest this region is integral to the processing of error detection and decision monitoring. Such functions have variously been interpreted as monitoring conflict (Carter et al 1998; Kiehl et al 2000a) and as a comparator making use of corollary discharge or efference copy (Coles et al 1995; Dehaene and Cohen 1994; Ghering et al 1993).

0006-3223/03/$30.00
doi:10.1016/S0006-3223(03)00419-0

There are now many studies from several neuroimaging domains (including positron emission tomography (PET), functional magnetic resonance [MR] imaging, and event-related potential [ERP]) that indicate functional cingulate abnormalities in schizophrenia. These include reduced activation in the cingulate region during error-related ERP negativity (Kiehl et al 2000b), reduced activation in an error detection-related Stroop paradigm (Carter et al 1997; Nordahl et al 2001), impaired self-monitoring performance during a continuous performance task (Carter et al 2001), and impaired sustained attention detected during an auditory discrimination test (Cohen et al 1998). Moreover, this evidence for a cingulate-based deficiency in self-monitoring has been related to an inability to distinguish one's own internal thoughts from external events, thus furnishing a possible basis for hallucinations (Ford et al 2001; Frith et al 1995). Of particular note, cingulate volume deficits in structural MRI studies have been reported to be associated with hallucinations, reduced executive functions, psychomotor poverty, and negative symptoms (Ashton et al 2000; Liddle et al 1992; Noga et al 1995; Paillere-Martinot et al 2001; Szeszko et al 2000). Taken together, these findings support the hypothesis that abnormalities in the cingulate region may play an important role in the pathophysiology of schizophrenia.

Of further note, all of the neuroimaging and anatomic studies suggest that proper function of the cingulate gyrus critically depends on connections with other parts of the neuronal network, rather than simply an sich (e.g., Benes 1993). In this article, we test the hypothesis that at least part of the abnormal activation and symptom association of the cingulate gyrus observed in patients with schizophrenia may, in fact, be attributed to disrupted white matter connections of this region with other limbic structures.

To date, white matter findings in schizophrenia have been equivocal. Most of the studies investigating volumetric differences between schizophrenia and control subjects have detected gray but not white matter volume reductions (see review by Shenton et al 2001). Two recent studies, however, both report white matter volume deficits, within the temporal and frontal regions on the left side (Sigmudsson et al 2001), and within the frontal lobes bilaterally (Paillere-Martinot et al 2001). Lack of compelling evidence for white matter abnormalities in schizophrenia is not surprising, given that white matter appears fairly uniform and homogeneous on conventional MR scans, where the orientation, density, and asymmetry of the fiber tracts cannot be visualized or quantified.

In contrast to structural MRI studies, diffusion tensor imaging (DTI; Basser 1995) represents an MRI technique that can detect subtle white matter abnormalities in vivo by assessing the degree to which directionally organized tissues have lost their normal integrity.[1] This method has recently been used to discern the directionality of white matter tracts in the normal human brain (Mamata et al, in press; Pierpaoli and Basser 1996) and to evaluate white matter fiber integrity in multiple sclerosis (Filippi et al 2001), stroke (Zelaya et al 1999), and Alzheimer's disease (Rose et al 2000). Of note, the first three studies in schizophrenia to quantify anisotropic diffusion showed lower anisotropy within prefrontal white matter (Buchsbaum et al 1998), as well as in whole white matter (Lim et al 1999) and in the splenium of the corpus callosum (Foong et al 2000b) relative to control subjects. A more recent study by our group (Kubicki et al 2002) showed a difference in asymmetry in the uncinate fasciculus, the white matter tract connecting the frontal and temporal lobe. Here, patients with schizophrenia did not show a left $>$ right asymmetry as was observed in normal control subjects. None of these studies, however, focused on the CB, as proposed here.

In this study, we apply line scan diffusion imaging (LSDI; Gudbartsson et al 1996) to obtain fractional anisotropy (FA) maps in 16 schizophrenia and 18 control comparison subjects to measure the integrity of fibers within the CB, the most prominent fiber tract within the limbic system. We also examined neuropsychologic correlates of FA of the CB in patients with schizophrenia. Based on prior studies that have linked CB to deficits in executive functions of performance monitoring in patients with schizophrenia, we examined correlations between our DTI measures and performance indices of the Wisconsin Card Sorting Test (WCST), a well-known neuropsychologic measure of executive functions.

Methods and Materials

Subjects

Sixteen patients with chronic schizophrenia **(**all men with a mean duration of illness of 22 years) were recruited from inpatient, day

[1] This technique is based on sensitizing the magnetic resonance signal to the movement of water and then determining the magnitude and direction of the water diffusion in three dimensions. In white matter, diffusion perpendicular to the direction of axons is restricted by both the myelin sheath and cell membrane such that diffusion will be greater along the length of the axon than perpendicular to it. In contrast, in cerebro-spinal fluid (CSF) and gray matter, water diffusion is equal in all directions. This characteristic of the diffusion (differing according to the medium's relative orientation) is termed anisotropy. The estimation of the diffusion tensor, a mathematical entity that characterizes water diffusion in all directions, has been proposed as an effective measure of diffusion in anisotropic tissue. In addition, several scalar measures describing the extent to which diffusion is anisotropic, indicating the presence of directional bias of the diffusion, have been proposed, the most popular being fractional anisotropy (FA) and relative anisotropy (RA) indices (Basser 1995), with the FA having a slightly higher signal-to-noise ratio than the RA index (Papadakis et al 1999). Higher anisotropy as measured by these indices indicates greater directionality and coherence of the fiber tracts, and lower anisotropy indicates less directionality of water in all directions measured.

treatment, outpatient, and foster care programs at the Veteran's Administration (VA) Boston Healthcare System, Brockton, MA. The Structured Clinical Interview for DSM-IV (SCID)—Patient Edition was used for diagnoses and the SCID-Nonpatient Edition (First 2001) interviews were completed for the 18 normal comparison subjects (also all men). The patient interviews were conducted by a clinically and research-trained psychiatrist or psychologist (MF or MES), and the normal comparison interviews were conducted by trained research assistants. Reliability for diagnoses is checked every 6 months and the Kappa coefficient has been greater than .90.

Comparison subjects were recruited from the general community and group-matched to patients on age, gender, handedness (Oldfield 1971), and parental socioeconomic status (Hollingshead 1965). Inclusion criteria for all subjects were right-handedness, age between 18 and 55 years, no history of electroconvulsive shock treatment, no history of neurologic illness, no alcohol or drug abuse in the previous 5 years, no medication with known effects on MR such as steroids, verbal IQ above 75, and an ability and desire to cooperate with the procedures as evidenced by written informed consent. The study was approved by both VA Boston Healthcare System and Harvard Medical School internal review board committees. All patients were receiving antipsychotic medication during the time of the MR scan. Four patients were receiving typical antipsychotics (fluphenazine and haloperidol), and nine patients were receiving atypical antipsychotics (clozapine, risperidone, and olanzapine). All medication doses were converted to chlorpromazine equivalence, and then these figures were used in all subsequent correlational analysis.

In addition, normal comparison subjects were screened to exclude individuals who had a first-degree relative with an Axis I disorder. We also excluded any persons with a history of substance abuse in the last year or dependence at any time.

Neuropsychologic Measures

We specifically selected the WCST as a means to examine the relationship of CB and executive function in patients with schizophrenia. Prior studies have suggested that one aspect of executive functioning, error monitoring, might be highly dependent on the integrity of the CB (e.g., Carter et al 1998; Cohen et al 1998; Nordahl et al 2001). The WCST provides measures of perseverative errors and perseverative responses, which may be viewed as indices of error monitoring, which in turn may be, as hypothesized here, correlated with reduced FA of the CB in patients with schizophrenia.

The WCST consists of 128 response cards (two decks, 64 cards per deck), each of which has a geometric feature that may vary along three dimensions (color, form, number). Subjects place each response card under one of four stimulus cards presented horizontally from the subject's field of view of left to right. The first stimulus card depicts a red triangle, the second two green stars, the third yellow crosses, and the fourth four blue circles. Subjects must deduce the correct sorting principle on the basis of performance feedback of "right" or "wrong" that is given to the subject after each trial. Cards are sorted first by color, followed by form, then by number, with each category requiring 10 consecutive correct responses. The sequence is then repeated. The dependent measures are number of incorrect responses, perseverative errors, nonperseverative errors, and number of categories achieved. As a control task, we also included a test of motor speed and dexterity, the Finger Tapping Test, which we predicted would not be associated with CB anisotropy measures.

MRI Protocol

All subjects were scanned using LSDI, a technique that can be implemented on conventional MR scanners.[2] The MR scans were performed with a quadrature head coil on a 1.5-Tesla GE Echospeed system (General Electric Medical Systems, Milwaukee, Wisconsin), which permits maximum gradient amplitudes of 40 mT/m. We began with a set of three orthogonal T1-weighted (T1W) images used as localizers (sagittal, axial oblique aligned to the anterior commissure–posterior commissure (AC-PC) line and another sagittal oblique aligned to the interhemispheric fissure). From the last sagittal oblique T1W image, the LSDI sequence in the coronal orientation was then aligned to be perpendicular to the AC-PC line. For each line, six images with high (1000 sec/mm^2) diffusion-weighting along six noncollinear directions were collected. For low (5 sec/mm^2) diffusion-weighting, we collected only two images, because diffusion related signal changes are minimal. The following scan parameters were used: rectangular field of view (FOV) 220 $\times$ 165 mm; 128 $\times$ 128 scan matrix (256 $\times$ 256 image matrix); slice thickness = 4 mm; interslice distance = 1 mm; receiver bandwidth $\pm$ 4 kHz; echo time = 64 msec; effective repetition time = 2592 msec; scan time = 60 sec/slice section. We acquired 31–35 coronal slices covering the entire brain, depending on brain size. The total scan time was 31–35 min. After reconstruction, the diffusion-weighted images were transferred to a SUN workstation, where eigenvalue, eigenvector, trace (TR), and FA maps of the diffusion tensor were calculated. Motion-related artifact maps were also constructed.

Methods of Quantification and Statistical Analysis

The portion of the CB that contains the most dense concentration of parallel fibers lies dorsal to the body of the corpus callosum. Throughout its extent, fibers of variable length join the bundle, and branches form connections between prefrontal and temporal regions in more anterior portions and between parietal and temporal regions in more posterior regions. The curving geometry of the CB, along with the branching of the fibers, prohibits reliable measurement of anisotropy in the most anterior and posterior portions, and for this reason we focused our analyses in the portion of the tract dorsal to the body of the corpus callosum. This measurement consisted of 8 coronal slices starting caudally with the first coronal slice posterior to the genu of the corpus

[2] Unlike the single-shot echo planar imaging (Turner et al 1990) and navigated echo pulsed gradient spin echo imaging (Ordidge et al 1994), the most commonly used magnetic resonance diffusion imaging techniques, line scan diffusion imaging, is composed of a series of parallel columns lying in the image plane. The sequential collection of these line data in independent acquisitions makes the sequence largely insensitive to bulk motion artifact, because no phase encoding is used, and shot-to-shot phase variations are fully removed by calculating the magnitude of the signal.

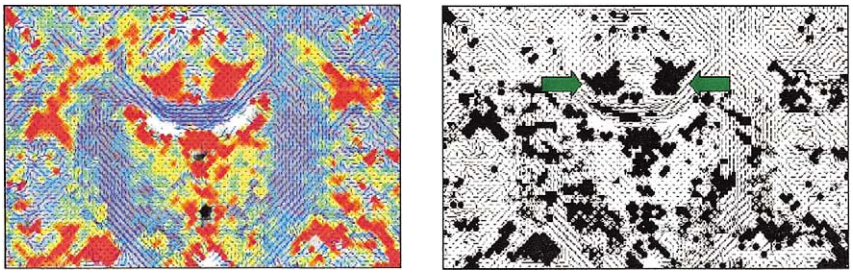

Figure 1. Left image represents diffusion tensor image of one of the eight slices used for the measurements. Right side illustrates the threshold applied to the diffusion tensor images, to define the cingulum bundle (arrows, see description in the text).

callosum. The resulting 40-mm section of the CB covered most of the fibers dorsal to the body of the corpus callosum.

The CB on all eight slices is perpendicular to the acquisition plane and is easy to detect on FA maps. To detect and quantify the CB, we used a method that was already successfully applied to the measurements of diffusion within the uncinate fasciculus in schizophrenia (Kubicki et al 2002). For all cases and all slices, a point centered within each fiber tract (separately for left and right side) was selected on the FA coronal map. Next, to extract this fiber tract from the neighboring structures, segmentation was defined by the maximum diffusivity (which for the cingulate fasciculus corresponds to the diffusion in the direction perpendicular to the coronal plane, parallel to the CB; $1 \times 10^{-}$ mm^2/sec was used as the fixed threshold for all the cases in both groups), and it was applied to each slice separately. Finally, a Matlab algorithm was used to display only voxels that survived this threshold criterion and were clustered with the point previously selected (Figure 1).

Next, we used the diffusion data to calculate three separate measures that were meant to describe as completely as possible the nature of white matter abnormalities in schizophrenia. Because our acquisition was two-dimensional and the segmentation based on the intensity did not produce smooth boundaries of the fiber tract, we averaged all our measures over eight consecutive slices to avoid the issue of interslice variability. First, we calculated anisotropic diffusion within the extracted CB. This measure, described in more detail later, depends on many factors, including the orientation and organization of the fiber tracts, their density, and the degree of myelination. We also calculated area of the extracted CB, because we were interested in the extent to which anisotropy differences between groups reflect the degree of connectivity between the frontal and temporal lobes (i.e., greater area in presence of equal anisotropy should represent more interconnecting fiber tracts). Finally, we calculated the trace of the diffusion tensor, TR, within the extracted CB. Because the trace is proportional to the mean diffusion (mean diffusivity = $(\lambda_1 + \lambda_2 + \lambda_3,) / 3 = TR / 3$), it depends on the strength of the diffusion (similar to the FA measure), but unlike the FA it does not reflect the restriction or organization of the diffusion. Thus, this measure theoretically can be used to rule in or rule out differences between groups that result from fiber density within the CB. More specifically, if the groups differed in FA and TR, then differences between groups in fiber density should be considered as the main factor related to group differences. If, however, FA but not TR is different between groups, then differences in orientation and organization of the fiber tracts rather than their density should be considered as the primary factor related to group differences. Mean FA, trace of diffusion, and mean area of the segmented fiber tract from the eight consecutive slices were calculated using following equations (Papadakis et al 1999), and measurements were done blind to diagnostic group:

$$FA = \frac{\sqrt{(\lambda 1 - \lambda 2)^2 + (\lambda 2 - \lambda 3)^2 + (\lambda 1 - \lambda 3)^2}}{\sqrt{2}\ \sqrt{\lambda 1^2 + \lambda 2^2 + \lambda 3^2}}$$

$$TR = \lambda_1 + \lambda_2 + \lambda_3 \qquad \text{(Papadakis et al 1999)}$$

where λ_1, λ_2, and λ_3 are three principal eigenvalues of the diffusion tensor. Thus we had three measures: 1) mean area of CB, 2) mean FA, and 3) mean trace of diffusion.

In addition, we calculated the degree of motion-related artifacts within the segmented region of interest. This measure was defined as the number of line segments that exhibited, on average, 30% or more signal loss when compared with neighboring line segments.

Repeated-measures analysis of variance (ANOVA) with group as a between-subjects factor and side as a within-subjects factor, were used to test for group differences in CB diffusion. In the case of a significant group effect, independent planned *t* tests were used to compare group differences, separately for the right and left hemispheres. Associations between neuropsychologic measures and diffusion measures were evaluated using Spearman rank-order correlations. In cases in which correlations were significant only on one side, or if they were significant in only one group of subjects, we used the Fisher's Exact Test to evaluate the differences between these correlations.

Results

Demographic Data

There were no group differences in age, handedness, or gender (Table 1). Groups did differ, however, in education and socioeconomic status, likely reflecting a decline in functioning in schizophrenia. Importantly, groups did not differ in parental socioeconomic status. Also, whereas verbal IQ showed differences between groups, a proxy measure of verbal IQ, oral reading of the Wide Range Achievement Test, version III (WRAT-III) (Wilkinson 1993), which is often considered a valid measure of premorbid IQ (Lezak 1995), did not show differences between groups.

Table 1. Sample Characteristics

	Schizophrenia Subjects (n = 16)	Control Subjects (n = 18)
Sex Ratio (% male)	100	100
Age	43 (6.8)	43 (5.9)
Education	12 (2.17)[a]	16 (2.22)
Socioeconomic Status (SES)	4.26 (.7)[a]	2.15 (1.09)
Parental SES	2.6 (1.18)	2.45 (1.19)
Handedness	.84 (.17)	.78 (.16)
Score of Minimental Status	28.53 (1.3)	29.1 (.79)
Verbal IQ	86.07 (13.67)[a]	110.6 (9.5)
WRAT-III Reading Score	51.2 (4.78)	47.7 (8.74)
Age of Onset	21.66 (3.3)	—
Chlorpromazine Equivalent of Neuroleptic Dose	464 (348)	—

Numbers in parentheses represent standard deviation.
SES, socioeconomic class; WRAT-III, Wide Range Achievement Test, version III.
[a] $p < .05$

All schizophrenic subjects were receiving neuroleptic medications, although none of the diffusion measures were correlated with estimates of chlorpromazine equivalences (i.e., rho = .33, p = .23 for right FA; rho = .22, p = .43 for left FA; rho = .27, p = .33 for right area; rho = .13, p = .65 for left area).

Anisotropy Measures

Both control and schizophrenic subjects showed a left >right asymmetry within the CB on all measures used [side effect; $F(1,33) = 37$; p = 3.5e-007].

ANOVA demonstrated effects for both areas of CB [$F(1,33) = 24$; p = 2.5e-005; Figure 2], as well as FA [$F(1,33) = 37$; p = 3.5e-007], but there were no statistically significant group-by-side interactions for any of the measures [FA: $F(1,33) = .14$; p = .71; CB: $F(1,33) = .44$; p = .55; Figure 3). More specifically, patients had smaller mean area and lower mean FA relative to control subjects. In post hoc analyses, independent sample t tests conducted separately for the right and left sides revealed a group difference in the area of the CB on the right (p = .05) and left (p = .05) sides, as well as lower mean diffusion anisotropy within the fiber tract on the right (p = .05) and left (p = .04) sides. Mean diffusivity, or trace of diffusion (TR), did not show group differences [$F(1,33) = 2.0$; p = .165].

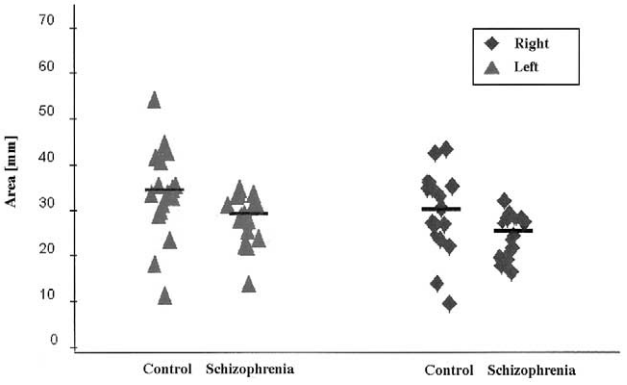

Figure 2. Mean area of the cingulum bundle (significant group × side interaction [$F(1,33) = 4.2$; p = .049]), followed by significant left ($p < .05$) and right ($p < .05$) group differences. Includes 16 subjects with chronic schizophrenia and 18 control subjects.

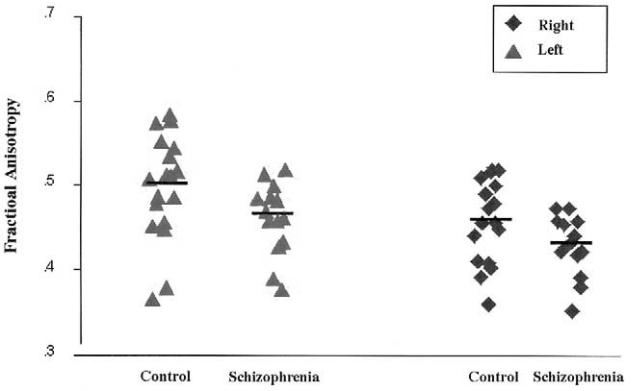

Figure 3. Mean fractional anisotropy within the cingulum bundle (significant group × side interaction [$F(1,33) = 4.6$; p = .039]), followed by significant left ($p < .04$) and right ($p < .05$) group differences. Includes 16 subjects with chronic schizophrenia and 18 control subjects.

Movement artifacts were detected on several slices in both groups; however, none of the subjects showed movement artifact on more than 3 slices. As the algorithm for analyzing diffusion data replaces the missing line segments with interpolated data from neighboring line segments, we believe that none of the measures were affected. Nevertheless, we analyzed the number of missing line segments between groups and found no statistically significant differences [$F(1,33) = 1.9$; p = .175].

Neuropsychologic Measures

For patients but not control subjects, Spearman rho correlations revealed significant associations between low scores on neuropsychologic performance measures and reduced CB fractional anisotropy. That is, reduced left CB FA correlated significantly with increased number of incorrect responses (rho = −.546, p = .04) and nonperseverative errors (rho = −.658, p = .01) on the WCST and approached significance for WCST perseverative errors (rho = −.521, p = .056). In addition, the Fisher's Exact Test confirmed the statistically significant left lateralization of the above correlations in schizophrenics. Here

correlations between left CB FA and neuropsychologic measures were compared with the correlations between right CB FA and the same neuropsychologic measures in schizophrenia ($Z = 2.06$; $p = .02$ for increased number of incorrect responses, $Z = 1.79$; $p = .04$ for nonperseverative errors, and $Z = 1.82$; $p = .03$ for perseverative errors). We also used the Fisher's Exact Test to compare correlation differences between left CB FA and neuropsychologic measures between the two groups. Here, statistically significant correlation differences were demonstrated between groups on the left side ($Z = 1.76$; $p = .04$ for increased number of incorrect responses, $Z = 2.28$; $p = .01$ for nonperseverative errors, and $Z = 1.92$; $p = .03$ for perseverative errors). The control task (Finger Tapping) results did not correlate significantly with any diffusion measures (FA left rho = .216 [$p = .438$]; FA right rho = .313 [$p = .256$]; area left rho = −.151 [$p = .590$]; area right rho = .090 [$p = .750$]) for either group.

Discussion

Our study revealed decreased diffusion anisotropy within the CB in male patients diagnosed with chronic schizophrenia compared with male normal comparison subjects. There are many studies suggesting a disruption in connectivity in schizophrenia (e.g., Fletcher et al 1999; Friston and Frith 1995; McGuire and Frith 1996; Weinberger et al 1992). Most of these studies point to a frontotemporal or temporolimbic network as the most likely pathway for these disruptions (Weinberger et al 1992; Yurgelun-Todd et al 1996); however, to our knowledge this is the first study to measure this interconnecting fiber tract in vivo.

The CB, as mentioned previously, is the most prominent white matter fiber tract of the limbic system. It underlies the cingulate gyrus and remains the only communication route between cingulate cortex and other areas of the brain, including prefrontal, parietal, temporal areas, and the thalamus (Domesick 1970). In this study, the decrease in diffusion anisotropy, as well as the decreased size of the fiber tract in schizophrenia, suggest a significant abnormality in the integrity of the fiber tracts interconnecting limbic structures.

Of note, a decrease in diffusion anisotropy as seen in our study can in theory result from any number of underlying physiologic causes. Direct DTI physiology experiments demonstrate that diffusion is restricted (anisotropic) in both myelinated and nonmyelinated fibers (Beaulieu and Allen 1994) and that the degree of diffusion anisotropy in white matter increases during myelination processes (Baratti et al 1999; Huppi et al 1998) and decreases in demyelination processes (Filippi et al 2001). In addition, Papadakis et al (1999) showed a relationship between fibers perpendicular to the main bundle of the fiber tract and an increase in noise along with a decrease in anisotropy measured within the fiber tract. Thus, pathologic processes that are responsible for the observed anisotropy findings could include decreased number or density of axons, decreased degree of myelination of the axons, decreased coherence of the fiber tract, or increased number or density of tracts perpendicular to the measured tract.

Which of these candidate pathologic processes can explain such changes in cingulate regions in schizophrenia? Findings from Benes et al point to a decrease in neuronal density in anterior cingulate cortex (Benes et al 1986, 2001) as well as an increase in the number of vertical neurons coming out of the cingulate gyrus (Benes et al 1992). If the vertical axons within the anterior cingulate gyrus were fibers leading to the thalamus, then they would have crossed perpendicular to the main bundle of the CB. These fibers could cause a decrease in anisotropy, as reported here.

It is also possible that the CB fiber tracts are less myelinated in schizophrenic patients than in comparison subjects. According to Benes et al (1994), myelination in specific brain regions (especially frontal and temporal lobes, where myelination still occurs in the second decade of life) might play a neuroprotective role, particularly with respect to the timing and appearance of symptoms in individuals at risk for schizophrenia. Indirect in vivo evidence for decreased myelination in frontal lobe white matter in schizophrenia comes also from an MR magnetization contrast study by Foong et al (2000a), although the CB has not been studied in particular. In addition, recent studies using both electron microscopy (Uranova et al 2001) and DNA analysis (Hakak et al 2001) reveal pathology of oligodendroglia cells in schizophrenia, cells that form the myelin sheaths.

We believe that results obtained using the diffusion measure of TR, shed some light on the issue of what group differences observed in our study between patients and controls in FA might mean. More specifically, in contrast to FA measures, the trace measures the average of the diffusion in all directions, thereby being more sensitive to fiber density than to fiber orientation and organization. Thus, if the FA decreases observed in schizophrenia were accompanied by increases in TR, this would suggest decreases in fiber density within the fiber tract. Our results, however, showed no differences between groups in TR, suggesting that the decreases in FA observed between groups are, at least in part, the result of differences between groups in the orientation or organization of the fiber tracts. It thus is likely that abnormalities in the orientation or organization of fiber tracts play a major role in schizophrenia white matter pathology.

Neuropsychologic performance measures correlated in the expected direction with decreased CB diffusion in patients with schizophrenia. Poorer scores on neuropsychologic measures, such as the WCST, which involve a set of complex mental operations related to set switching, inhibiting a dominant response, mental tracking and sequencing, and categorization, correlate with diffusion abnormalities.

These mental operations are heavily dependent on prefrontal and anterior cingulate gyrus, which together may form a neural network important to many of the diverse executive functions of working memory (Cohen et al 2000). Consistent with these findings are those that patients with schizophrenia also show decreased activation in dorsolateral prefrontal and anterior cingulate regions while engaged in neuropsychologic measures that tap these diverse executive functions of working memory (e.g., Carter et al 2001; Weinberger 1988). Taken together, this DTI study and prior functional studies raise the interesting question of whether working memory impairment in schizophrenia might be related not only to prefrontal and cingulate abnormalities, but also to reduced CB fibers that serve to connect these regions. Reduced connectivity of a dorsolateral prefrontal and anterior cingulate network in schizophrenia could lead to a disturbance in the efficient coordination of mental operations that constitute the executive division of working memory. Moreover, abnormal anterior cingulate modulation of the prefrontotemporal integration, demonstrated by Fletcher et al (1999) using PET and working memory test and confirmed with another type of statistical analysis (canonical variates analysis of patterns of "functional connectivity") by Meyer-Lindenberg et al (2001), could be the result of abnormalities occurring within the fiber tracts interconnecting these brain regions in schizophrenia.

Other DTI studies, despite some methodologic differences (see the review by Kubicki et al 2002) also report anisotropy alterations in schizophrenia. Lim et al (1999), for example, using a measure of FA averaged over large regions of interest, reported widespread loss of anisotropy in schizophrenic compared with control subjects. Buchsbaum et al (1998), using methods implementing statistical probability maps, reported decreased relative anisotropy within left prefrontal white matter regions in schizophrenia. A similar approach was also used by another group of investigators (Agartz et al 2001), revealing FA reductions within the splenium of the corpus callosum in patients with chronic schizophrenia compared with control subjects. Of note, Foong et al (2000b), using a region of interest approach, reported loss of FA within the splenium but not in the genu of the corpus callosum in patients with schizophrenia compared with control subjects. A recent study from our laboratory (Kubicki et al 2002) also revealed diminished left–right FA asymmetry in uncinate fasciculus in schizophrenic patients compared with control subjects, a finding suggesting that frontotemporal connections might be aberrant in schizophrenia.

Taken together, these findings provide strong evidence for loss of integrity within the white matter fiber tracts in schizophrenia. The question remains, however, as to whether there is a widespread pathologic process affecting all white matter or whether the process is more localized. Another question that remains to be addressed is whether the anomaly is due to any one, or more, of the pathologic processes described within the white matter fiber tracts or whether such an anomaly is secondary to regional alterations within gray matter areas interconnected by the fiber tract. Such questions can only be addressed with further studies, including DTI techniques that use in vivo fiber tractography to model the major white matter fiber tract pathways in the brain (Basser et al 2000).

We note there are several limitations of our study. First, the sample size is relatively small, although previous diffusion studies revealed similar results on smaller populations. Nonetheless, the small sample size may restrict the generalization of these findings to the larger group of patients diagnosed with schizophrenia. Second, our populations included men only. Other studies report schizophrenia-related differences in the distribution and especially in the asymmetry of gray matter that are gender specific (see, for example, Takahashi et al [2002]). Thus, findings from our study can only be generalized to male schizophrenic patients, and further studies that include women should be conducted to characterize further anatomic abnormalities in schizophrenia. Third, the area measurement used in our study is derived from the segmentation based on the maximum diffusivity. Thus, if this maximal diffusivity was smaller in schizophrenia, the area of the CB would have been smaller also. In other words, it is possible that the area differences could be driven by the diffusion differences within the structure, and thus not reflect the actual difference in the fiber area. Fourth, it is possible that there is the same number of fibers belonging to the CB in both patients with schizophrenia and control subjects, but these fibers are dispersed over a greater area in the patient group, with their density not high enough to survive the applied threshold. If this is the case, however, then the trace—that is, the sum of diffusion in all three directions—should show a relative, and even a more significant, increase, which was not the case in our study. Our data thus indicate that in schizophrenia, disorganization and lower coherence rather than lower density of the fibers plays a major role. Fifth, all patients were diagnosed with schizophrenia and medicated, although medication dosage did not correlate with DTI measures. It will be important, nonetheless, in future

studies, to evaluate patients who are medication naive to rule out the effects of neuroleptics on DTI measures. Sixth, neuropsychologic and DTI associations were based on multiple correlations, although, as predicted, a control measure of motor speed and dexterity failed to correlate with CB abnormalities.

Seventh, a further limitation is that the extent of our measurement did not cover the entire CB. Without histologic verification, the origin and termination of fibers measured in our study is not precisely known. This region of CB does, however, contain fibers interconnecting anterior cingulate gyrus with prefrontal (dorsolateral prefrontal), medial dorsal thalamus, temporal areas, and posterior cingulate gyrus, as well as fibers connecting posterior cingulate gyrus with frontal regions and direct connections between dorsolateral prefrontal cortex and parahippocampal gyrus and hippocampus (Goldman-Rakic et al 1984; Vogt et al 1992). Thus, the variety of functions driven by this fiber tract might limit the possible findings of correlations between diffusion measures and clinical symptoms.

Additionally, the method used limits the number of fiber tracts that can be evaluated only to those that are perpendicular to the plane of acquisition. Hence, future studies using different, more robust segmentation methods are needed to address the question of the localized versus more generalized white matter pathology in schizophrenia. Finally, future longitudinal studies are needed to examine the stability of these DTI findings in patients with schizophrenia as well as the stability of the correlations with neuropsychologic variables and symptoms.

In summary, this study introduces a new approach to evaluating white matter fiber tracts in patients diagnosed with schizophrenia. Our study lends evidence to the notion that there is a disruption in brain connectivity in schizophrenia, and it points to the cingulate bundle as one possible source of this disruption.

This study was supported by the National Alliance for Research on Schizophrenia and Depression (MK, MF), the National Institute of Health (Grant Nos. K02 MH 01110 and R01 MH 50747 to MES, R01 MH 40799 to RWM, and R01 NS 39335 to SEM), the Department of Veterans Affairs Merit Awards (MES, PGN, RWM), the National Center for Research Resources (11747 to RK and P41 1321 to FAJ and CFW), and a VA Psychiatry/Neuroscience Research Fellowship Award (MF).

We thank Marie Fairbanks for her administrative assistance.

References

Agartz I, Andersson JL, Skare S (2001): Abnormal brain white matter in schizophrenia: A diffusion tensor imaging study. *Neuroreport* 12:2251–2254.

Ashton L, Barnes A, Livingston M, Wyper D (2000): Cingulate abnormalities associated with PANSS negative scores in first episode schizophrenia. *Behav Neurol* 12:93–101.

Baratti C, Barnett AS, Pierpaoli C (1999): Comparative MR imaging study of brain maturation in kittens with T1, T2, and the trace of the diffusion tensor. *Radiology* 210:133–142.

Basser PJ (1995): Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. *NMR Biomed* 8:333–344.

Basser PJ, Pajevic S, Pierpaoli C, Duda J, Aldroubi A (2000): In vivo fiber tractography using DT-MRI data. *Magn Reson Med* 44:625–32.

Beaulieu C, Allen P (1994): Determinants of anisotropic water diffusion in nerves. *Magn Reson Med* 31:394–400.

Benes FM (1993): Relationship of cingulate cortex to schizophrenia and other psychiatric disorders. In: Vogt BA, Gabriel M, editors. *Neurobiology of Cingulate Cortex and Limbic Thalamus: A Comprehensive Handbood.* Boston: Birkhauser, 581–605.

Benes FM, Davidson J, Bird ED (1986): Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. *Arch Gen Psychiatry* 43:31–35.

Benes FM, Turtle M, Khan Y, Farol P (1994): Myelination of a key relay zone in the hippocampal formation occurs in the human brain during childhood, adolescence, and adulthood. *Arch Gen Psychiatry* 51:477–484.

Benes FM, Vincent SL, Alsterberg G, Bird ED, SanGiovanni JP (1992): Increased GABAA receptor binding in superficial layers of cingulate cortex in schizophrenics. *J Neurosci* 12:924–929.

Benes FM, Vincent SL, Todtenkopf M (2001): The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. *Biol Psychiatry* 50:395–406.

Bouras C, Kovari E, Hof PR, Riederer BM, Giannakopoulos P (2001): Anterior cingulate cortex pathology in schizophrenia and bipolar disorder. *Acta Neuropathol (Berl)* 102:373–379.

Buchsbaum MS, Tang CY, Peled S, Gudbjartsson H, Lu D, Hazlett EA, et al (1998): MRI white matter diffusion anisotropy and PET metabolic rate in schizophrenia. *Neuroreport* 9:425–430.

Carter CS, Braver TS, Barch DM, Botvinick MM, Noll D, Cohen JD (1998): Anterior cingulate cortex, error detection, and the online monitoring of performance. *Science* 280:747–749.

Carter CS, MacDonald AW 3rd, Ross LL, Stenger VA (2001): Anterior cingulate cortex activity and impaired self-monitoring of performance in patients with schizophrenia: An event-related fMRI study. *Am J Psychiatry* 158:1423–1428.

Carter CS, Mintun M, Nichols T, Cohen JD (1997): Anterior cingulate gyrus dysfunction and selective attention deficits in schizophrenia: [15O]H2O PET study during single-trial Stroop task performance. *Am J Psychiatry* 154:1670–1675.

Cohen JD, Botvinick M, Carter CS (2000): Anterior cingulate and prefrontal cortex: Who's in control? *Nat Neurosci* 3:421–423.

Cohen RM, Nordahl TE, Semple WE, Andreason P, Pickar D (1998): Abnormalities in the distributed network of sustained attention predict neuroleptic treatment response in schizophrenia. *Neuropsychopharmacology* 19:36–47.

Coles MG, Scheffers MK, Fournier L (1995): Where did you go wrong? Errors, partial errors, and the nature of human information processing. *Acta Psychol (Amst)* 90:129–144.

Crespo-Facorro B, Kim J, Andreasen NC, O'Leary DS, Bockholt HJ, Magnotta V (2000): Insular cortex abnormalities in schizophrenia: A structural magnetic resonance imaging study of first-episode patients. *Schizophr Res* 46:35–43.

Dehaene S, Cohen L (1994): Dissociable mechanisms of subitizing and counting: Neuropsychological evidence from simultanagnosic patients. *J Exp Psychol Hum Percept Perform* 20:958–75.

Domesick VB (1970): The fasciculus cinguli in the rat. *Brain Res* 20:19–32.

Filippi M, Cercignani M, Inglese M, Horsfield MA, Comi G (2001): Diffusion tensor magnetic resonance imaging in multiple sclerosis. *Neurology* 56:304–311.

First MB, Spitzer RL, Gibbon M, Williams JB (2001): *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Nonpatient Edition.* New York: Biometrics Research Department, New York State Psychiatric Institute, 67–71.

Fletcher P, McKenna PJ, Friston KJ, Frith CD, Dolan RJ (1999): Abnormal cingulate modulation of fronto-temporal connectivity in schizophrenia. *Neuroimage* 9:337–342.

Foong J, Maier M, Barker GJ, Brocklehurst S, Miller DH, Ron MA (2000a): In vivo investigation of white matter pathology in schizophrenia with magnetisation transfer imaging. *J Neurol Neurosurg Psychiatry* 68:70–74.

Foong J, Maier M, Clark CA, Barker GJ, Miller DH, Ron MA (2000b): Neuropathological abnormalities of the corpus callosum in schizophrenia: A diffusion tensor imaging study. *J Neurol Neurosurg Psychiatry* 68:242–244.

Ford JM, Mathalon DH, Heinks T, Kalba S, Faustman WO, Roth WT (2001): Neurophysiological evidence of corollary discharge dysfunction in schizophrenia. *Am J Psychiatry* 158:2069–2071.

Friston KJ, Frith CD (1995): Schizophrenia: A disconnection syndrome? *Clin Neurosci* 3:89–97.

Frith CD, Friston KJ, Herold S, Silbersweig D, Fletcher P, Cahill C, et al (1995): Regional brain activity in chronic schizophrenic patients during the performance of a verbal fluency task. *Br J Psychiatry* 167:343–349.

Ghering WJ, Goss B, Coles MGH, Meyer DE, Donchin E (1993): A neural system for error detection and compensation. *Psychol Sci* 4:385–390.

Goldman-Rakic PS (1988): Topography of cognition: Parallel distributed networks in primate association cortex. *Annu Rev Neurosci* 11:137–156.

Goldman-Rakic PS, Selemon LD, Schwartz ML (1984): Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. *Neuroscience* 12:719–743.

Goldstein JM, Goodman JM, Seidman LJ, Kennedy DN, Makris N, Lee H, et al (1999): Cortical abnormalities in schizophrenia identified by structural magnetic resonance imaging. *Arch Gen Psychiatry* 56:537–547.

Gudbartsson H, Maier S, Mulkern R (1996): Line scan diffusion imaging. *Magn Reson Med* 36:509–519.

Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, et al (2001): Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc Natl Acad Sci U S A* 98:4746–4751.

Hollingshead A (1965): *Two Factor of Index of Social Position.* New Haven: Yale Station.

Huppi PS, Maier SE, Peled S, Zientara GP, Barnes PD, Jolesz FA, Volpe JJ (1998): Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. *Pediatr Res* 44:584–590.

Kiehl KA, Liddle PF, Hopfinger JB (2000a): Error processing and the rostral anterior cingulate: An event-related fMRI study. *Psychophysiology* 37:216–223.

Kiehl KA, Smith AM, Hare RD, Liddle PF (2000b): An event-related potential investigation of response inhibition in schizophrenia and psychopathy. *Biol Psychiatry* 48:210–221.

Kubicki M, Westin CF, Maier SE, Frumin M, Nestor PG, Salisbury DF, et al (2002): Uncinate fasciculus findings in schizophrenia: A magnetic resonance diffusion tensor imaging study. *Am J Psychiatry* 159:813–820.

Kubicki M, Westin CF, Maier SE, Mamata H, Frumin M, Ersner-Hershfield H, et al (2002): Diffusion tensor imaging and its application to neuropsychiatric disorders. *Harv Rev Psychiatry* 10:324–336.

Lezak M (1995): *Neuropsychological Assessment.* New York: Oxford University Press.

Liddle PF, Friston KJ, Frith CD, Frackowiak RS (1992): Cerebral blood flow and mental processes in schizophrenia. *J R Soc Med* 85:224–227.

Lim KO, Hedehus M, Moseley M, de Crespigny A, Sullivan EV, Pfefferbaum A (1999): Compromised white matter tract integrity in schizophrenia inferred from diffusion tensor imaging. *Arch Gen Psychiatry* 56:367–374.

Mamata H, Mamata Y, Westin CF, Shenton ME, Kikinis R, Jolesz FA, Maier SE (2002): High-resolution line-scan diffusion-tensor MRI of white matter fiber tract anatomy. *AJNR* 23:67–75.

McGuire PK, Frith CD (1996): Disordered functional connectivity in schizophrenia [editorial]. *Psychol Med* 26:663–667.

Meyer-Lindenberg A, Poline JB, Kohn PD, Holt JL, Egan MF, Weinberger DR, Berman KF (2001): Evidence for abnormal cortical functional connectivity during working memory in schizophrenia. *Am J Psychiatry* 158:1809–1817.

Noga JT, Aylward E, Barta PE, Pearlson GD (1995): Cingulate gyrus in schizophrenic patients and normal volunteers. *Psychiatry Res* 61:201–208.

Nordahl TE, Carter CS, Salo RE, Kraft L, Baldo J, Salamat S, et al (2001): Anterior cingulate metabolism correlates with stroop errors in paranoid schizophrenia patients. *Neuropsychopharmacology* 25:139–148.

Oldfield RC (1971): The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 9:97–113.

Ordidge RJ, Helpern JA, Qing ZX, Knight RA, Nagesh V (1994): Correction of motional artifacts in diffusion-weighted MR images using navigator echoes. *Magn Reson Imaging* 12:455–460.

Paillere-Martinot M, Caclin A, Artiges E, Poline JB, Joliot M, Mallet L, et al (2001): Cerebral gray and white matter reductions and clinical correlates in patients with early onset schizophrenia. *Schizophr Res* 50:19–26.

Pandya DN, Seltzer B (1982): Intrinsic connections and architectonics of posterior parietal cortex in the rhesus monkey. *J Comp Neurol* 204:196–210.

Papadakis NG, Xing D, Houston GC, Smith JM, Smith MI, James MF, et al (1999): A study of rotationally invariant and symmetric indices of diffusion anisotropy. *Magn Reson Imaging* 17:881–892.

Pierpaoli C, Basser PJ (1996): Toward a quantitative assessment of diffusion anisotropy. *Magn Reson Med* 36:893–906.

Rose SE, Chen F, Chalk JB, Zelaya FO, Strugnell WE, Benson M, et al (2000): Loss of connectivity in Alzheimer's disease: An evaluation of white matter tract integrity with colour coded MR diffusion tensor imaging. *J Neurol Neurosurg Psychiatry* 69:528–530.

Shenton ME, Dickey CC, Frumin M, McCarley RW (2001): A review of MRI findings in schizophrenia. *Schizophr Res* 49:1–52.

Sigmudsson T, Suckling J, Maier M, Williams S, Bullemore ET, Greenwood KE (2001): Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. *Am J Psychiatry* 158:234–243.

Szeszko PR, Bilder RM, Lencz T, Ashtari M, Goldman RS, Reiter G, et al (2000): Reduced anterior cingulate gyrus volume correlates with executive dysfunction in men with first-episode schizophrenia. *Schizophr Res* 43:97–108.

Takahashi T, Kawasaki Y, Kurokawa K, Hagino H, Nohara S, Yamashita I, et al (2002): Lack of normal structural asymmetry of the anterior cingulate gyrus in female patients with schizophrenia: A volumetric magnetic resonance imaging study. *Schizophr Res* 55:69–81.

Turner R, Le Bihan D, Maier J, Vavrek R, Hedges LK, Pekar J (1990): Echo-planar imaging of intravoxel incoherent motion. *Radiology* 177:407–414.

Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001): Electron microscopy of oligodendroglia in severe mental illness. *Brain Res Bull* 55:597–610.

Vogt BA, Finch DM, Olson CR (1992): Functional heterogeneity in cingulate cortex: The anterior executive and posterior evaluative regions. *Cereb Cortex* 2:435–443.

Vogt BA, Rosene DL, Pandya DN (1979): Thalamic and cortical afferents differentiate anterior from posterior cingulate cortex in the monkey. *Science* 204:205–207.

Weinberger DR (1988): Schizophrenia and the frontal lobe. *Trends Neurosci* 11:367–370.

Weinberger DR, Berman KF, Suddath R, Torrey EF (1992): Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: A magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. *Am J Psychiatry* 149:890–897.

Wilkinson GS (1993): *Wide Achievement Test*, 3rd ed. Wilmington, DE: Wide Range Inc.

Yurgelun-Todd DA, Waternaux CM, Cohen BM, Gruber SA, English CD, Renshaw PF (1996): Functional magnetic resonance imaging of schizophrenic patients and comparison subjects during word production. *Am J Psychiatry* 153:200–205.

Zelaya F, Flood N, Chalk JB, Wang D, Doddrell DM, Strugnell W, et al (1999): An evaluation of the time dependence of the anisotropy of the water diffusion tensor in acute human ischemia. *Magn Reson Imaging* 17:331–348.