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Age Effects on Diffusion Tensor Magnetic Resonance Imaging Tractography Measures of Frontal Cortex Connections in Schizophrenia

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Abstract: Diffusion tensor magnetic resonance imaging (DT-MRI) has previously been used to investigate white matter tracts in schizophrenia, with inconsistent results. The aim of the study was to use a novel method for tract-specific measurements of fronto-temporal fasciculi in early-onset schizophrenia. We hypothesized that by making tract-specific measurements, clear diffusion abnormalities would be revealed in specific fasciculi in schizophrenia. Measurements of diffusion anisotropy and mean diffusivity were localized within fronto-temporal fasciculi by forming 3-D reconstructions of the cingulum, uncinate, superior longitudinal, and inferior fronto-occipital fasciculi using diffusion tensor tractography. We were limited in our ability to test our hypothesis by the important and surprising finding that age affected DT-MRI-based measures in schizophrenia patients in a different way from comparison subjects, most notably in the left superior longitudinal fasciculus. The youngest schizophrenia patients that we studied had lower diffusion anisotropy than age-matched comparison subjects, but this difference diminished with increasing age. The main conclusion of this study was that direct comparisons of absolute DT-MRI-based measures between individuals with schizophrenia and comparison subjects may be problematic and misleading because of underlying age-related differences in brain maturation between groups. *Hum Brain Mapp 27:230–238, 2006.* © **2005 Wiley-Liss, Inc.**

Key words: MRI; fasciculi; white matter; anisotropy; mean diffusivity

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INTRODUCTION

A disturbance of functional connectivity, the integrated operation of topographically distinct brain regions, has been proposed as the cardinal "lesion" in schizophrenia [Friston and Frith, 1995]. However, methods for assessing white matter fasciculi in vivo (and hence for examining a substrate of connectivity directly) have previously been unavailable. One promising technique for studying changes in specific tracts is diffusion tensor magnetic resonance imaging (DT-MRI) [Basser et al., 1994]. However, studies of patients with schizophrenia using DT-MRI [Agartz et al., 2001; Buchsbaum et al., 1998; Burns et al., 2003; Foong et al., 2000, 2002; Kubicki et al., 2002; Lim et al., 1999; Steel et al., 2001] have reported inconsistent results, with some groups reporting reduced anisotropy in schizophrenics compared with comparison subjects [Agartz et al., 2001; Buchsbaum et al., 1998; Burns et al., 2003; Foong et al., 2000; Kubicki et al., 2002; Lim et al., 1999] and others finding no significant differences [Foong et al., 2002; Steel et al., 2001]. This may relate to the variety of analysis methods employed—in particular the localization of white matter regions on which anisotropy measures are based. The corpus callosum is a large, easy to identify structure and hence is amenable to study [Agartz et al., 2001; Foong et al., 2000], and a combination of visual inspection and reference to Talairach coordinates has been used to localize measures within the uncinate, superior longitudinal fasciculus, and the cingulum [Burns et al., 2003; Kubicki et al., 2002]. Since white matter fasciculi are often long and tortuous 3-D structures, any analytic approach based on identification of a tract from a single image slice or point will inevitably result in large portions of target tract data being missed. Further, if the location of putative tract abnormalities is restricted to a particular portion of a fiber, there is also a good chance that this may be missed by a single plane cross-sectional approach.

Recently, the observation that the axis of fastest water diffusion is parallel to the dominant fiber orientation in vivo [Moseley et al., 1990a] led to the development of diffusion tensor "tractography," in which the 3-D pathways of white matter tracts are reconstructed by sequentially piecing together discrete and closely spaced estimates of fiber orientation to form continuous trajectories [e.g., Basser et al., 2000; Conturo et al., 1999; Mori et al., 1999]. We used tractography in a novel approach to study the brains of 14 patients with schizophrenia and 14 appropriately age-matched healthy subjects. Our aim was to localize measurements of mean diffusivity and diffusion anisotropy within specific candidate tracts along as much of their course as could be visualized by tractography. Evidence from functional imaging and neuropsychological studies in schizophrenia indicates that abnormalities of the functional connectivity of the frontal lobes accompany the disorder [Fletcher et al., 1999; Ford et al., 2001; Frith et al., 2000; Jennings et al., 1998; Meyer-Lindenberg et al., 2001]. We therefore hypothesized that measures of mean diffusivity and diffusion anisotropy made within the major white matter tracts that form connections to the frontal cortex (the uncinate fasciculus, the superior longitudinal fasciculus (SLF), the inferior fronto-occipital fasciculus (IFO), and the cingulum) [Dejerine, 1895; Crosby et al., 1962] would be abnormal in schizophrenia. This work has previously been reported in abstract format in Jones et al. [2003a,b]

SUBJECTS AND METHODS

Subjects

Schizophrenia subjects

Fourteen right-handed males with DSM-IV criteria schizophrenia were recruited from wards and clinics at the South London and Maudsley NHS Trust, London. Their median age was 34 years (SD = 9.3; range = 22–53 years) and their median IQ, measured using the National Adult Reading Test [Nelson 1991], was 110 (range = 98–124). The median illness duration was 8 years (range = 1–25 years) and all were in remission and being treated with antipsychotic medication. Exclusion criteria included history of head injury, neurological symptoms, speech or hearing difficulties, fulfillment of DSM-IV criteria for abuse of illicit drugs or alcohol during their lifetime, and any contraindications to MRI scanning, including metal implants and claustrophobia.

Comparison subjects

Fourteen right-handed male comparison subjects with a median age of 34 years (SD = 9.8; range = 19–57 years) and a median IQ of 109 (range = 99–123) were recruited. All comparison subjects had no medical/psychiatric disorders, no family history of psychiatric disorder, and were not receiving medication. There were no significant differences between patient and comparison subjects in relation to the mean age ($t = 0.32$, df = 26, $P = 0.75$).

Subjects from both groups gave written consent after the procedure had been fully explained. The study was approved by the local research Ethics Committee. As part of our standard clinical protocol, each subject was scanned with conventional (T_1- and T_2-weighted) scans and these scans were examined for gross abnormalities by a neuroradiologist. If any subject had exhibited such abnormalities, they would have been excluded from the study.

Data Acquisition

Data were acquired using a GE Signa 1.5 T LX MRI system (General Electric, Milwaukee, WI) and an acquisition optimized for diffusion tensor MRI of white matter, providing whole head coverage with isotropic image resolution (2.5 × 2.5 × 2.5 mm). The acquisition was peripherally gated to the cardiac cycle using a plethysmograph placed on the subjects' forefingers. Full details are provided in Jones et al. [2002]. Following correction for image distortions introduced by the diffusion-weighting gradients, the diffusion tensor was determined in each voxel [Basser et al., 1994].

Fiber-Tracking

Measurements of diffusion anisotropy and mean diffusivity were localized to specific fasciculi using the technique of "fiber-tracking" or "tractography" [Basser et al., 2000; Catani et al., 2002]. This technique, by following the pathway of least hindrance to the diffusion of water molecules, aims to create 3-D reconstructions of white matter tracts non-invasively. Full details of the tractography method employed in the current study are provided elsewhere [Basser et al., 2000; Catani et al., 2002] but a brief description follows.

First, locations for the initiation of the tracking algorithm (referred to here as "seedpoints") were defined. By cross-referencing neuroanatomical works [Crosby et al., 1962; Dejerine, 1895], one of the authors (M.C.) who was blind to individual subjects' group status, defined 3-D regions of interests (ROIs) believed to contain a section of the desired fasciculus on an image showing fractional anisotropy [Basser and Pierpaoli, 1996] (a diffusion tensor-derived quantitative measure of water diffusion directionality on a scale from 0–1, with 0 corresponding to isotropic diffusion, i.e., no preferred orientation, and 1 corresponding to the case where diffusion occurs only along one axis). At each seedpoint the fiber orientation was determined (from the diffusion tensor) and the tracking algorithm moved a distance of 0.5 mm along this direction. The diffusion tensor and hence fiber orientation was determined at the new location and the algorithm moved a further 0.5 mm along this direction. A pathway was traced out in this manner until the fractional anisotropy fell below an arbitrary threshold (set to 0.15). The procedure was then repeated by tracking from the seedpoint in the opposite direction to the first step in order to reconstruct the whole tract passing through the seedpoint. One ROI was defined for fasciculi whose boundaries were clearly delineated on the fractional anisotropy image. In tracts such as the superior longitudinal fasciculus and cingulum, there are no other fasciculi in the neighborhood of their central portions and it was straightforward to define a single ROI to include only fibers belonging to that particular tract. However, in regions where fasciculi run closely to one another (e.g., the uncinate and inferior fronto-occipital fasciculi), definition of a single ROI that includes the fibers of only one of the fasciculi is difficult. To overcome this problem, a second ROI was defined at a distance from the first ROI, such that it contained at least a section of the desired fasciculus but no fibers of the undesired fasciculi. Only those pathways that were launched from the first ROI and passed through the second ROI were retained for analysis [Catani et al., 2002; Conturo et al., 1999].

Visualization of Tracts

Trajectories were checked by qualitative visual assessment (by M.C.) for consistency with neuroanatomical texts [Crosby et al., 1962; Dejerine, 1895] by reconstructing them in three dimensions. A set of polygons with fixed radius and circular cross-section was generated to connect the points forming the trajectory and lighting was added to give a 3-D computerized image of the fasciculi [Catani et al., 2002].

Deriving Tract-Specific Measurements

As it was necessary to determine the diffusion tensor at the end of each incremental (0.5 mm) step in the reconstruction of tracts in order to determine the fiber orientation (and hence the direction in which to take the next step), it was possible to determine the mean diffusivity and fractional anisotropy at regular (0.5 mm) intervals along the fasciculus of interest. These measures were stored as the tracking process continued, and at the termination of tracking the average fractional anisotropy and average mean diffusivity for the particular tract were determined. In this way, measurements of anisotropy and mean diffusivity were confined to within the fasciculus of interest.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS v. 12.0; SPSS, Chicago, IL). Age differences between the two subject groups were investigated by the use of a *t*-test for independent samples. The study design meant that eight measures of fractional anisotropy (FA) and mean diffusivity (MD) were made within each subject (four fasciculi, each with right- and left-sided measures). As these measures are potentially highly interrelated, it was necessary to use a repeated measures model to examine analysis of covariance (ANCOVA). Each ANCOVA was designed to incorporate within-subject factors (FA or MD measures in all eight tracts) and between-subject factors (membership of patient or comparison group). Since it has been reported that there is an age-related dependence of various indices of diffusion within the adult brain [Engelter et al., 2000; Gideon et al., 1994; Nusbaum et al., 2001; O'Sullivan et al., 2001; Pfefferbaum et al., 2000; Virta et al., 1999], age was included as a covariate in the above analyses. An alpha level of 0.05 was used to denote significance. When a significant main effect (age or group) or a significant interaction (Age × Group) was revealed, the repeated measures model allowed us to examine these effects in relation to the eight individual tracts (presented as a *t*-statistic and *P*-value). Preliminary reports of the methodology appear elsewhere [Jones et al., 2003a,b].

RESULTS

Tractography and Tract-Specific Measurements

Tract maps of the uncinate, superior longitudinal, and inferior fronto-occipital fasciculi and the cingulum within the right and left hemispheres were successfully constructed in all 14 schizophrenia patients and all 14 comparison subjects. Figure 1 shows an example of reconstructed trajecto-

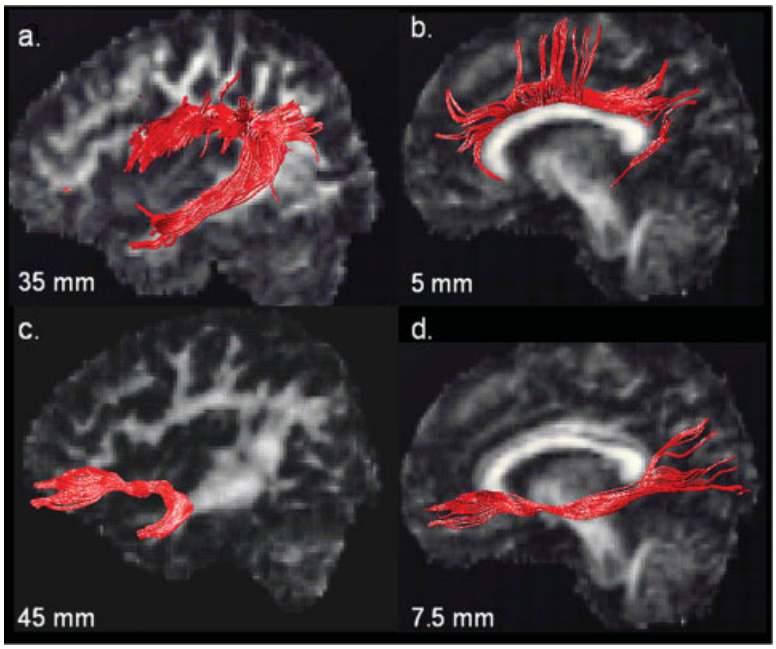

Figure 1.
Typical reconstructions of major association fasciculi of the left frontal lobe in a schizophrenic patient (**a**) the superior longitudinal fasciculus (SLF); (**b**) the cingulum bundle; (**c**) the uncinate fasciculus; and (**d**) the inferior fronto-occipital fasciculus (IFO). The backdrop shows the fractional anisotropy image (distance from the mid-line is given in mm).

ries of the candidate white matter tracts that constitute the longitudinal association fiber system in the left hemisphere of a single patient with schizophrenia. Reference to standard anatomical texts [Crosby et al., 1962; Dejerine, 1895] shows that these reconstructions closely match descriptions obtained from postmortem studies [Catani et al., 2002]. The superior longitudinal fasciculus connects posterolateral prefrontal cortex (Broca's area) with other language areas of the parietal and temporal lobe (including Wernicke's area). The cingulum extends from the paraolfactory area of Broca to the hippocampus passing around the corpus callosum. The uncinate fasciculus connects orbital and polar prefrontal cortex with the anterior part of the temporal lobe and the inferior fronto-occipital fasciculus connects infero- and dorsolateral frontal cortex with posterior temporal cortex and the occipital lobe.

Statistical Analysis

Surrogate markers of tract volume

The number of seedpoints used to reconstruct a trajectory and the total number of steps taken to reconstruct a trajectory might be expected to be dependent on the tract volume. However, for each fasciculus (SLF, Uncinate, IFO, and Cingulum), there were no significant differences in the number of seedpoints nor in the total number of steps between patients and comparison subjects.

Fractional anisotropy (FA)

A repeated-measures ANCOVA revealed a significant effect of group membership ($F = 9.22$, df = 1, 24, $P = 0.006$) and an interaction of Group × Age ($F = 5.59$, df = 1, 24, $P = 0.03$). When the effect of subject group was considered, FA measures (for all eight tracts) were lower among patients (mean = 0.405, SE = 0.004, CI = 0.396–0.413) than comparison subjects (mean = 0.422, SE = 0.004, CI = 0.414–0.431) (Fig. 2). When individual tracts were examined, only differences in FA measures in the left superior longitudinal fasciculus (SLF) achieved statistical significance ($t = 4.15$, $P < 0.0001$) with values that were lower in patients than controls. When the Age × Group interaction was considered, age was significantly and negatively correlated with mean FA (within all eight tracts) in comparison subjects ($r = -0.53$, $P = 0.049$), but showed no such significant association with mean FA in the patient group ($r = 0.37$, $P = 0.194$) (Fig. 3). An examination of each of the different tracts showed the extent of the interaction of Age × Group only to be significant in the left superior longitudinal fasciculus ($t = -3.31$, $P = 0.003$): there was a highly significant negative correlation between FA measures in the left SLF and age in comparison subjects ($r = -0.71$, $P = 0.005$), which was not present in patients ($r = 0.43$, $P = 0.12$).

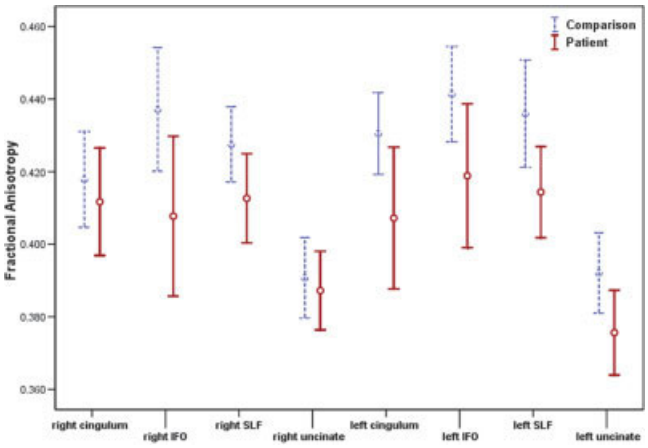

Figure 2.

Mean (95% confidence intervals) fractional anisotropy measurements obtained from right- and left-sided tracts (cingulum, inferior fronto-occipital fasciculus (IFO), superior longitudinal fasciculus (SLF) and uncinate fasciculus) in comparison subjects and patients.

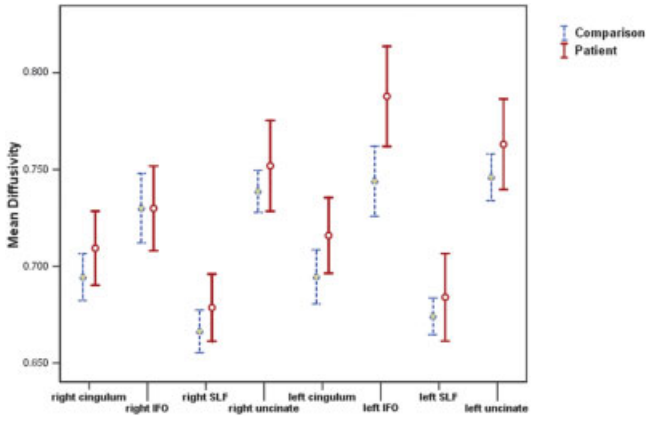

Figure 4.

Mean diffusivity measurements (95% confidence intervals) from right- and left-sided tracts (cingulum, inferior fronto-occipital fasciculus, superior longitudinal fasciculus, and uncinate fasciculus) in comparison subjects and patients.

Mean diffusivity

A repeated-measures ANCOVA revealed a main effect of group membership (F = 5.82, df = 1, 24 $P = 0.024$) and an interaction of Age × Group which approached statistical significance (F = 4.19, df = 1, 24, $P = 0.052$). When the main effect of group was examined, mean MD (for all eight tracts) measures were found to be higher among patients (mean = 0.727, SE = 0.007, CI = 0.713–0.740) than comparison subjects (mean = 0.711, SE = 0.007, CI = 0.697–0.724) (Fig. 4). There were no statistically significant differences between the subject groups in relation to individual tract MD measures. When the Age × Group interaction was examined, there was a trend towards a negative correlation in patients ($r = -0.52$, $P = 0.059$) but not comparison subjects ($r = 0.14$, $P = 0.63$) (Fig. 5).

DISCUSSION

Tract-Specific Measurements

Earlier applications of DT-MRI to schizophrenia have examined white matter either in a nonlocalized manner or within ROIs in tracts identified by visual inspection. Some studies have been unable to demonstrate anisotropy differences from healthy comparison subjects [Foong et al., 2002; Steel et al., 2001], other studies found changes that have been impossible to precisely localize in the complex anatomy of cerebral white matter [Buchsbaum et al., 1998; Lim et al.,

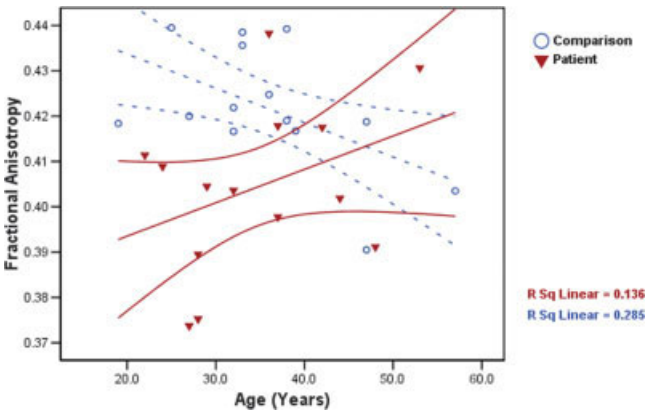

Figure 3.

Fractional anisotropy (mean of all eight tracts (95% confidence intervals)) vs. age in comparison subjects and patients. The r^2 values of the regression lines describe the proportion of variance in FA (y) contributed to by age (x) for each group.

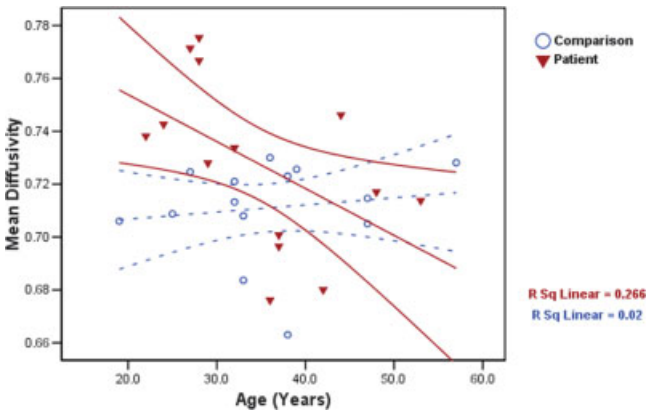

Figure 5.

Mean diffusivity, mean of all eight tracts (95% confidence intervals), vs. age in control subjects and patients. The r^2 values of the regression lines describe the proportion of variance in FA (y) contributed to by age (x) for each group.

1999], and other studies have been able to localize changes within tracts that are easy to identify [e.g., Foong et al., 2000, found changes in the corpus callosum]. We reasoned that some of the DT-MRI discrepancies in the schizophrenia literature might originate from inconsistencies in accurate identification/localization of the structures being investigated. It is well known that diffusion anisotropy is highly variable in white matter and it is likely that imperfect localization may significantly affect the results. To mitigate this problem, we performed tract-specific diffusion measurements in which tracts were identified by DT-MRI tractography. To our knowledge, this study represents the first time that such an approach has been applied to the investigation of schizophrenia. Compared to the conventional approach of taking measurements from manually drawn ROIs, our method presents several advantages: (1) it allows us to investigate specific tracts that are hypothesized to be involved in the pathogenesis of schizophrenia; (2) it provides data from the entire tract rather than from a limited portion of it; and (3) it is less prone to operator-induced bias.

There are two further issues which we wish to discuss in relation to using tractography to elucidate differences in diffusion characteristics. First, as described in Subjects and Methods, tracking was terminated when the anisotropy fell below a given threshold (FA = 0.15). This may introduce a bias in the measurements, but this bias would ensure that the statistical analysis would err on the side of being extremely conservative. If one assumes a tract in the patients to have lower anisotropy than in the control group, one might expect the tract reconstruction to progress less far into low anisotropy regions in the patient group than in the control group. A possible consequence would be overestimation of FA values in the group with reduced FA. Clearly, a bias of this type will not invalidate the statistics but ensures that they are conservative and that any difference observed is likely to be genuine. Second, it is possible that the branching behavior may be somewhat different between subjects (something that we did not test in this study). The fact that peripheral regions of the tracts are likely to have lower anisotropy than in the trunk means that it could be argued that tracts with more branching may have different diffusion measurements than those with less branching. To the best of our knowledge, there is no technique currently extant for robust quantification of "branching" from DT-MRI data. Consequently, we are unable at this stage to determine how much of the patient–control differences we report are due to this phenomenon, but this is certainly a topic worthy of further investigation.

We chose to focus our investigation on the major tracts that connect frontal with lateral and medial cortex, particularly those connected to language areas in the left hemisphere, hypothesizing that they would show structural abnormalities in schizophrenia. These are relatively long association fibers whose growth and extended maturation in the brain during fetal life and childhood make them plausible targets for the substrate of a neurodevelopmental disorder. Of these tracts, the superior longitudinal fasciculus, which contains large numbers of fibers connecting Broca's and Wernicke's areas, was a prime candidate for the investigation of abnormalities in patients with schizophrenia. Indeed, a recently published DT-MRI study in which anisotropy measurements were taken in ROIs in superior longitudinal fasciculus implicated this structure in schizophrenia [Burns et al., 2003]. Similarly, there are DT-MRI reports of reduced fractional anisotropy in the uncinate fasciculus in schizophrenia [Kubicki et al., 2002; Burns et al., 2003].

Interpretation of Differences in Diffusion Characteristics

The interpretation of any differences in measured diffusion anisotropy, which quantifies the degree to which the measured diffusivity depends on the direction in which it is measured, is not straightforward. It is important to bear in mind that DT-MRI estimates of diffusion anisotropy are obtained from within finite volumes (i.e., image voxels) and therefore represent a volume-averaged quantity. As the diameter on an axon is of the order of 10 μm, and DT-MRI data is typically acquired using voxels with dimensions on the scale of millimeters, it is clear that each voxel-averaged estimate of anisotropy is contributed to by thousands of axons. If all axons within a voxel are arranged in a highly parallel fashion, then the measured diffusion anisotropy will be very high. Conversely, if the ensemble of axons is arranged so that their orientations are evenly distributed in space, the measured anisotropy will be very low. Thus, the measured anisotropy of white matter is strongly dependent on its architectural paradigm [Pierpaoli et al., 1996]. It is therefore possible, in principle, for a loss of white matter fibers within a voxel in which the orientation of the axons is nonuniform to lead to an increase in the measured anisotropy. If the fibers that remain within the voxel are, on average, more uniformly oriented than the ensemble prior to the fiber loss, then the measured anisotropy will increase [Pierpaoli et al., 2001].

Furthermore, the degree to which diffusion is hindered in a direction perpendicular to the long-axis of the axonal bundle will depend somewhat on the integrity of the axonal membranes and the degree of myelination. If there is a challenge to the integrity of the axonal membranes, then hindrance in the direction perpendicular to the long-axis of the fibers will be reduced, leading to a reduction in measured anisotropy. Similarly, the diffusion anisotropy in a less myelinated bundle will be less than in a more heavily myelinated axonal bundle. Clearly, any finding of differences in anisotropy within fronto-temporal fasciculi between patients with schizophrenia and age-matched comparison subjects is open to a number of interpretations. The data would be consistent with either the presence of a different architectural paradigm for the axons within fronto-temporal fasciculi, differences in myelination of tracts, differences in the integrity of the axonal membranes between patients and comparison subjects, or a combination of such effects.

The interpretation of differences in mean diffusivity is also not straightforward. It is well documented that diffusion characteristics change in disease. For example, there is a sharp reduction in diffusivity during acute ischemia [Moseley et al., 1999b]. However, experimental evidence shows that diffusion characteristics also change in health throughout the life cycle. During the later stages of life there is an increase of diffusivity with increasing age [Engelter et al., 2000; Gideon et al., 1994; Nusbaum et al., 2001; O'Sullivan et al., 2001; Pfefferbaum et al., 2000; Sullivan et al., 2001; Virta et al., 1999] that is attributed to rarefaction of the white matter.

Age Effects

Our main finding is that age is a significant covarying factor when assessing water diffusivity in the fronto-temporal pathways in schizophrenia. Differences in fractional anisotropy between patients and comparison subjects were most pronounced in the youngest subjects and tended to disappear with increasing age. A similar trend was observed in relation to mean diffusivity measures.

In addition to the variability in spatial localization of the measurements mentioned above, this unexpected finding may explain inconsistencies of previous DT-MRI findings in schizophrenia. None of the previous DT-MRI studies in schizophrenia had included age as a possible covariate. If a standard statistical comparison between patient and comparison subjects is performed in the presence of a strong second-order interaction, results may differ depending on the age range of the subjects being investigated. Studies that include older schizophrenia patients are less likely to find differences between patients and control than studies that include very young subjects. Unfortunately, since this finding was unexpected our study was not designed to provide an interpretation of it. For example, we cannot separate the effect of age at time of scanning from the age at symptom onset. Within the patient group there was a statistically significant ($P = 0.012$) correlation between age at time of scanning and age at symptom onset (Fig. 6). Hence, the youngest patients also represent those with the earliest onset of schizophrenia. It could be speculated that patients with onset of schizophrenia in early adult life have more severe abnormalities (reduced fractional anisotropy) than those who do not develop psychosis until middle age. A second and more speculative interpretation relates to the observation that measures of diffusion anisotropy within white matter increase between birth and late adolescence as adult myelination patterns are established.

Similarly, mean diffusivity in the immature brain is higher than that in the adult brain, but this decreases between birth and adolescence [Huppi et al., 1998; Neil et al., 1998]. However, in neurodevelopment there is elevated diffusivity and reduced anisotropy compared to the mature adult brain at birth and a subsequent reduction in diffusivity and increase in diffusion anisotropy to adult values during the first two decades of life [Huppi et al., 1998; Mukherjee et al., 2001, 2002; Neil et al., 1998]. Consequently, the apparent increases

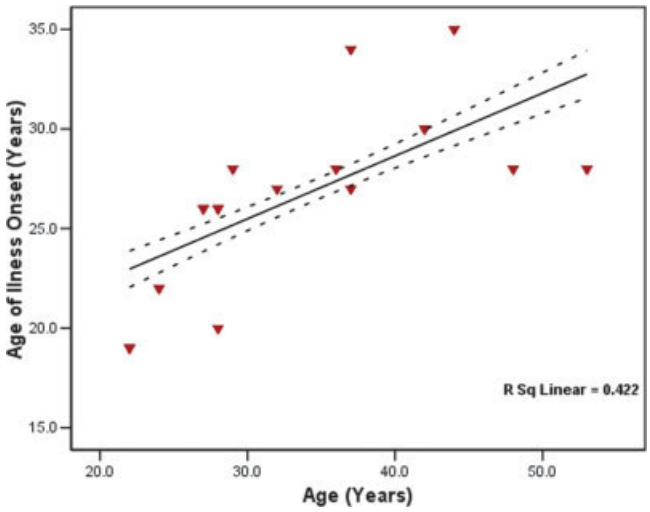

Figure 6.
Regression line (95% confidence interval) for age of onset of symptoms vs. age for patients with schizophrenia.

in fractional anisotropy and decreases in mean diffusivity with age seen in our group of schizophrenia patients could be interpreted as evidence that white matter maturational changes, completed by early adult life in health, are delayed and ongoing in schizophrenia. Whatever the underlying explanation for our results, the effects of age and age of onset of symptoms should be taken into account when performing diffusion tensor MRI studies of schizophrenic patients.

Mean diffusivity and fractional anisotropy are independent measures. For example, mean diffusivity is fairly homogeneous throughout parenchyma [Pierpaoli et al., 1996], while fractional anisotropy is extremely heterogeneous. Surprisingly, mean diffusivity has rarely been considered in studies of schizophrenia. Our findings suggest that this often-neglected measure should also be included in future DT-MRI studies of schizophrenia.

It was not possible, owing to the small sample size, to subdivide patients on the basis of clinical characteristics that may have potentially confounded our results. For instance, diffusion anisotropy have been recently found to be higher in the left cingulum of patients with schizophrenia who were experiencing auditory hallucinations than patients who were not [Hubl et al., 2004]. The majority (86%) of patients in our sample had a history of prominent auditory hallucinations, scoring 4 or more on the Brief Psychiatric Rating Scale [BPRS; Overall and Coram, 1962]. Thus, it is possible that the extent of the differences between patients and comparison subjects may have been underestimated, owing to relatively higher FA measures in the patient group associated with the presence of hallucinations.

We cannot exclude the possibility that group differences in FA may have been contributed to by antipsychotic exposure in the patient group, as all of the patients were prescribed regular antipsychotic medication; four patients were

receiving typical drugs and 10 patients were prescribed atypical drugs. However, white matter abnormalities have been recently described in first-episode patients with schizophrenia who had been receiving antipsychotic medication for only a few days [Woolley et al., 2004].

Finally, we note that this novel approach for extracting tract-specific measurements of diffusion characteristics produces an extremely large number of estimates from each bundle. In this study, we simply compared the mean of these values from each tract. This approach would dilute the effects of localized damage to white matter tracts should they exist. However, in schizophrenia—given the suspected pathophysiology—we do not expect focal lesions to be present. Of course, we are not suggesting that the technique we propose here will be suitable for the study of all diseases. For example, if severe localized damage exists, such as might arise from stroke or multiple sclerosis, one would have to reconsider using the technique proposed here. In such instances, it might be more appropriate to attempt to coregister the segmented tracts and attempt to get point-to-point correspondence between tracts. Of course, the method presented in this work does not preclude the user from continuing in this fashion, as it already provides the necessary segmented data.

Tractography has been used to segment pyramidal tracts for subsequent quantitative studies of multiple sclerosis [Wilson et al., 2003] and congenital hemiparesis [Glenn et al., 2003]. It is also conceivable that the technique proposed here may prove a useful tool for the further investigation of conditions in which DT-MRI has previously demonstrated differences on a more global basis, e.g., in studies of aging [Engelter et al., 2000; Gideon et al., 1994; Nusbaum et al., 2001; O'Sullivan et al., 2001; Pfefferbaum et al., 2000; Sullivan et al., 2001; Virta et al., 1999], IQ, and reading ability [Klingberg et al., 2000].

CONCLUSION

We have demonstrated the use of a tractographic approach to make quantitative measurements of fractional anisotropy within specific white matter tracts. Our results suggest that, at least in our cohort of subjects, diffusion abnormalities in the fronto-temporal tracts are most likely to be observed in very young patients. Future DT-MRI studies in schizophrenia should consider that "age" and/or "age at the onset of the disease" are important factors to be taken into account in the experimental design and data interpretation.

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