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The Paradoxical Relationship between White Matter, Psychopathology and Cognition in Schizophrenia: A Diffusion Tensor and Proton Spectroscopic Imaging Study

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White matter disruption has been repeatedly documented in schizophrenia consistent with microstructural disorganization (reduced fractional anisotropy (FA)) and axonal dysfunction (reduced N-acetylaspartate NAAc). However, the clinical significance of these abnormalities is poorly understood. Diffusion tensor and proton spectroscopic imaging where used to assess FA, axial diffusivity and radial diffusivity (RD), and supra-ventricular white matter NAAc, respectively, in 64 schizophrenia and 64 healthy subjects. Schizophrenia patients had reduced FA across several regions, with additional regions where FA correlated positively with positive symptoms severity. These regions included genu, body and splenium of corpus callosum, anterior and superior corona radiata, superior longitudinal and inferior fronto-occipital fasciculi, and internal capsule. The FA/symptoms relationships corresponded with opposite correlations between RD and positive symptoms. The schizophrenia group (SP group) had progressively reduced NAAc with age, and NAAc correlated negatively with positive symptoms. Cognition correlated positively with both FA and NAAc in controls, whereas in the SP group it had a negative correlation with NAAc and no significant relationship with FA. Antipsychotic dose did not account for the results. Correlates of psychosis, cognitive and negative symptoms can be found in white matter. The significant correlations between positive symptoms in schizophrenia and diffusion and NAAc measures suggest decreased axonal density with increased glial cells and higher myelination in this subpopulation. A separate set of abnormal relationships between cognition and FA/RD, as well as with NAAc, converge to suggest that in schizophrenia, white matter microstructure supports the two core illness domains: psychosis and cognitive/negative symptoms.
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INTRODUCTION

White matter (WM) abnormalities have been described in schizophrenia (SP) using morphometric, post mortem and diffusion tensor imaging (DTI; Hoistad *et al*, 2009). DTI studies find reduced fractional anisotropy (FA), a measure of water diffusivity consistent with myelination and/or axonal coherence abnormalities (Skudlarski *et al*, 2013). However, the topographical organization, time of development, diagnostic specificity, and neurobiological mechanism underlying the FA reductions are not clear. In addition, the psychopathological and cognitive correlates of FA in SP remain unknown.

More recently, it has been reported that in SP, higher FA values may correlate with psychotic symptom severity (ie hallucinations and delusions (Hubl *et al*, 2004)). This counterintuitive relationship (in the face of reduced FA) has been interpreted as increased structural connectivity among regions involved in language production and monitoring, with resultant misattribution of inner speech, leading to psychotic symptoms (Shergill *et al*, 2007). However, these studies involved small samples (10–34) and were vulnerable to selection bias of WM regions. Others have not found this correlation (Skelly *et al*, 2008).

We have examined the relationships between psychopathology and cognition with two measures of WM physiology (diffusion and axonal integrity) in the largest sample of SP and healthy control (HC) subjects. DTI was analyzed with an unbiased approach (Tract Based Spatial Statistics, TBSS (Smith *et al*, 2006)). Proton magnetic resonance spectroscopic imaging (^{1}H-MRSI) assessed *N*-acetylaspartate compounds (NAAc) in WM, a measure of axonal integrity. We hypothesized positive relationships between FA and positive symptoms. We also explored correlations between FA, radial and axial diffusivity (RD and AD, respectively), and cognitive measures, as well as with negative symptoms and between WM NAAc and cognition and psychopathology.

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Finally, we expected to detect FA (Hoistad *et al*, 2009) and NAAc (Kraguljac *et al*, 2012) reductions in SP.

MATERIALS AND METHODS

Subjects

Patients with SP were recruited from the University of New Mexico Hospitals and the Albuquerque Veterans-Affairs-Medical-Center. Inclusion criteria were: (i) DSM-IV-TR SP made through consensus by two research psychiatrists using the SCID-DSM-IV-TR, Patient-Version and (ii) clinically stable on the same antipsychotic medications >4 weeks. Exclusion criteria were: (i) diagnosis of neurological disorder; (ii) current substance-use disorder (except for nicotine); (iii) metallic implants; and (iv) claustrophobia. HC were excluded for any of the following: (i) any current DSM-IV-TR axis-I disorder (SCID-DSM-IV-TR Non-Patient-Version; (except current nicotine) or any past history of a disorder (except for substance use); and (ii) first-degree relative with psychotic disorder. The local IRB approved the study. Subjects provided informed consent and were paid.

Clinical and Neuropsychological Assessments

Subjects completed the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS; Buchanan *et al*, 2005). In patients, psychopathology was assessed with the Positive-and-Negative-Syndrome-Scale (PANSS; Kay *et al*, 1987). PANSS raters reached good inter-rater reliabilities (positive symptom ICC = 0.86 and negative symptom ICC = 0.64). Assessments were completed within 1 week of scanning.

MR Acquisition

A Siemens 3 T Tim-Trio with 12-channel-RF coil was used. An MPRAGE was initially acquired: 1.0 mm sagittal slices, 7° Flip angle, $T_R = 2530$ ms, $TE_1 = 1.64$ ms, $TE_2 = 3.5$ ms, $TE_3 = 5.36$ ms, $TE_4 = 7.22$ ms, $TE_5 = 9.08$ ms, FOV was 256 × 256. The DTI was obtained in the axial direction along the AC-PC line, had 30 directions, $b = 800$ s/mm^2 and five interleaved measurements of $b = 0$. The FOV was 256 × 256 mm with a 2 mm slice thickness, 72 slices, 128 × 128 matrix size, voxel size = 8 mm^3, TE = 84 ms, TR = 9000 ms, NEX = 1, partial Fourier encoding of 3/4, and with a GRAPPA acceleration factor of 2 (6 min total).

^{1}H-MRSI was performed with point-resolved spectroscopy sequence (PRESS). Briefly, PRESS with and without water pre-saturation were acquired (TE = 40 ms, TR = 1500 ms, slice thickness = 15 mm, FOV = 220 × 220 mm, circular k-space sampling (radius = 24), 20 min. total). The nominal voxel size was 6.9 × 6.9 × 15 mm^3 with effective volume of 2.4 cm^3. The one ^{1}H-MRSI slab was immediately above the lateral ventricles and parallel to AC-PC axis including portions of the medial frontal and parietal lobes (Gasparovic *et al*, 2006).

DTI Data Analysis

The analysis was based on FSL (available from: http://fsl.fmrib.ox.ac.uk). Preprocessing consisted of the following: (i) removal of gradient directions with signal dropouts owing to motion (subjects with $>10\%$ gradient directions removals were not included); (ii) motion and eddy current correction; and (iii) corrected gradient directions for any image rotation completed during the previous motion correction step. FA, RD and AD, scalar diffusion parameters, were calculated using DTIFIT. The FA image was normalized to MNI space with a nonlinear registration algorithm (FNIRT). A mean FA image was calculated from these spatially normalized images. The TBSS algorithm was then applied to the mean FA image to calculate a mean WM tract skeleton. The FA data of each subject was then projected onto the mean WM skeleton. Similar analyses were performed for RD and AD.

^{1}H-MRSI Data Analysis

^{1}H-MRSI data were analyzed using Linear-Combination-Model (Provencher, 1993). We automatically selected spectra with goodness-of-fit, as measured by Craemer-Rao-Lower-Bound of $\leqslant 20$ for *N*-acetylasparate plus NAAc, the metabolite of interest. These values were corrected for partial volume of gray matter (GM), WM and cerebrospinal fluid (using segmented T1 images) and relaxation effects, as outlined previously. Voxels with WM fraction (WM%÷(WM%+GM%)) $\geqslant 66\%$ were considered 'white matter' voxels. ('gray matter voxels' and other neuro-metabolite data will be included in a future report with the full sample). Finally, voxels were classified as right or left hemisphere and frontal or parietal based on their position relative to the central sulcus (see Gasparovic *et al*, 2006 for full description)

Statistical Analysis

FA. Statistical linear models were tested on the TBSS skeleton. Significance of model parameters with multiple comparison correction ($p = 0.05$) was determined by threshold-free cluster enhancement (TFCE; Smith and Nichols, 2009) combined with 5000 non-parametric permutations (randomize, FSL). Fifty regions on the skeleton were labeled as per a WM atlas (Mori *et al*, 2008). The number of voxels and their mean FA reaching statistical significance were calculated for each of these skeleton regions. Group differences in FA, accounting for main and interactive effects of age was tested with the formula:

$$FA_k = \alpha_0 HC_k + \alpha_1 SP_k + \alpha_2 (Age_k - 40) + \alpha_3 HC_k (Age_k - 40), \quad (1)$$

where k is indexed over subjects, FA_k is the FA at a voxel for k^{th} subject, $HC_k = 1$ for HC and $SP_k = 1$ for SP, and Age_k is the subject's age. The contrast α_0–α_1 was tested for the difference of FA between the two groups; α_2 is the slope of FA dependence on age (the selection of 40 to center age does not affect any conclusions). The interaction term α_3 examines different relationships for FA and age across the groups (α_3 was not significant, whereas α_2 was, hence we include age as a covariate for all further analyses).

The following model examined in SP the FA relationship with positive PANSS scores (α_4) accounting for antipsychotic dosage, as olanzapine equivalents (OLZ; Gardner *et al*, 2010); represented by α_5):

$$FA_k = \alpha_2 (Age_k - 40) + \alpha_4 Pscore_k + \alpha_5 OLZ_k \quad (2)$$

A similar analysis was done on negative PANSS scores.

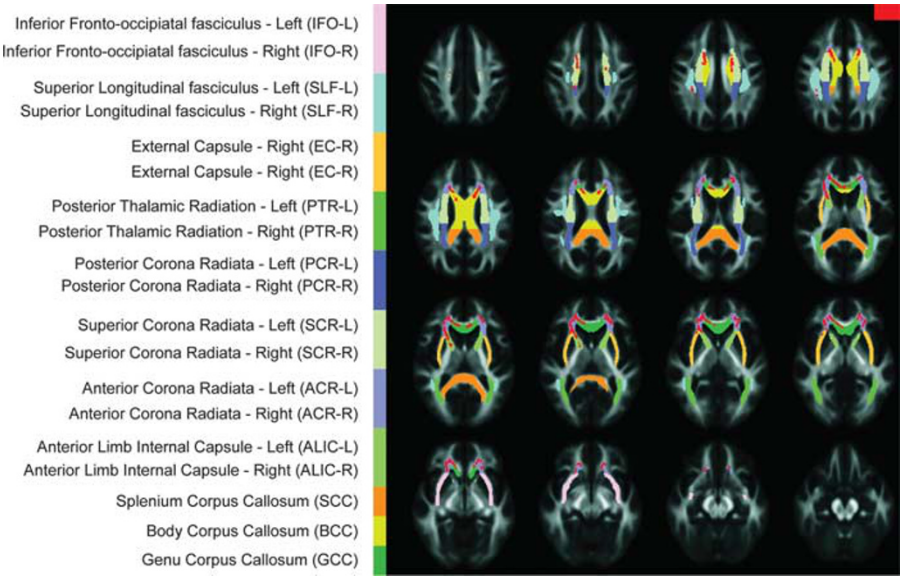

Figure 1 Spatial distribution of voxels with significantly reduced FA (in red) in schizophrenia ($n=64$) compared with healthy controls ($n=64$) accounting for age. Various colors identify some fasciculi (Mori *et al*, 2008).

Finally, the main effect of MATRICS overall score (α_6) and its interaction with group (α_7) was examined by the formula:

$$FA_k = \alpha_0 HC_k + \alpha_1 SZ_k + \alpha_2 (Age_k - 40) + \alpha_6 Matrics_k + \alpha_7 Matrics_k HC_k \quad (3)$$

Similar analyses were performed for RD and AD.

NAAc. NAAc values from all WM voxels were the dependent variable for PROC-MIXED (SAS version-9) analyses, with diagnosis as the grouping factor and age. MATRICS overall score, PANSS scores and OLZ were also used as covariates. Only significant interactions or main effects were followed with *post hoc* tests.

RESULTS

Demographics

Ninety SP and 74 HCs were studied. However, 26 SP and 10 HCs were excluded because of DTI artifacts (no subjects had defined brain abnormalities). Hence, 64 SP and 64 HCs were included (Supplementary Table). Of these, 64 SP and 61 HCs had ^{1}H-MRSI data. There were no significant differences between the groups in: age or socioeconomic status (SES) of the family of origin. The SP group had a lower proportion of females ($\chi^2=2.9$, $p=0.09$), a higher proportion of smokers ($\chi^2=4.3$, $p=0.04$) and worse personal SES ($\chi^2=25.7$, $p<0.0001$). Vascular risk factors (Jonckheere–Terpstra test, $p=0.02$) and history of cannabis ($\chi^2=7.9$, $p=0.0005$) and stimulant use ($\chi^2=5.4$, $p=0.02$) were greater in the SP group. Excluded subjects did not differ in demographic, clinical, or neuropsychological measures compared with the subjects studied (p-values >0.05).

Group Differences in Diffusion

SPs had lower FA across multiple brain regions compared to HCs at $p=0.05$ (TFCE; α_0–α_1 is significantly positive in equation (1), see Figure 1). The distribution of significant voxels is shown in the Table 1 (column B). Reduced FA was found in anterior corona radiata, genu and body of the corpus callosum, superior corona radiata, right anterior limb of the internal capsule, and superior longitudinal fasciculus. There were no regions where FA was significantly higher in SPs. Including gender and smoking status did not affect these differences. FA and AD decreased whereas RD increased with age but there were no interactions with diagnosis and AD and RD were not found to be significantly different between the two groups.

Diffusion and Symptomatology

The spatial distribution of significant correlations between FA and positive PANSS scores in SPs, based on equation (2) is shown in Figure 2a. FA had positive correlations with positive symptoms across multiple regions, whereas OLZ and negative symptoms had no effect. The Table 1 (column C) shows the distribution of significant voxels, several of which overlap with those having a group difference. They included: genu, body and splenium of corpus callosum, anterior, superior, and posterior corona radiata, superior longitudinal and inferior fronto-occipital fasciculi, and the internal capsule. FA averaged over these significant regions positively correlated with positive symptoms ($r_{62}=0.59$, $p<0.0001$; Figure 2b). RD had negative correlations with positive symptoms through many of the same regions as FA ($r_{62}=0.57$, $p<0.0001$; Figure 2c) wheras AD had no such relationships. There were no correlations between hallucinations severity and FA or RD across any regions.

Diffusion and Cognition

Over many voxels the MATRICS total score and group interacted in relationship to FA (α_7 with $P<0.05$ from equation (3); see Figure 3a and Table 1, column D). A similar interaction was found for RD but not for AD. In the regions

Table 1 Spatial Distribution of Voxels Across 50 Standardized Fasciculi (Mori *et al*, 2008)

	Volume (mm^3)	A (mm^3)	B (mm^3)	C (mm^3)	D (mm^3)	E (mm^3)	F (mm^3)	G (mm^3)
Middle cerebellar peduncle	2596	1	0	449	0	0	0	0
Pontine crossing tract	367	0	0	146	0	0	0	0
Genu—corpus callosum	1797	1768	632	1136	1040	984	0	207
Body—corpus callosum	3253	3129	507	1562	2097	2108	124	225
Splenium—corpus callosum	2335	1834	0	1220	1161	1546	15	0
Column and body of fornix	138	69	0	0	0	0	0	1
Corticospinal tract (R)	413	13	0	8	0	0	0	0
Corticospinal tract (L)	406	50	0	75	0	0	0	0
Medial lemniscis (R)	173	0	0	73	0	0	0	0
Medial lemniscus (L)	151	0	0	41	0	0	0	0
Inferior cerebellar peduncle (R)	181	0	0	65	0	0	0	0
Inferior cerebellar peduncle (L)	163	0	0	107	0	0	0	0
Superior cerebellar peduncle (R)	247	0	0	99	0	0	0	0
Superior cerebellar peduncle (L)	244	0	0	150	0	0	0	0
Cerebral peduncle (R)	619	467	0	261	0	0	0	0
Cerebral peduncle (L)	629	480	0	236	34	33	0	0
Anterior limb internal capsule (R)	818	574	249	475	0	212	0	45
Anterior limb internal capsule (L)	812	622	0	523	3	276	0	0
Posterior limb internal capsule (R)	923	507	2	232	0	1	0	0
Posterior limb internal capsule (L)	935	548	0	209	58	44	0	0
Retrolenticular internal capsule (R)	764	477	0	264	0	105	0	0
Retrolenticular internal capsule (L)	773	512	0	346	373	308	0	0
Anterior corona radiata (R)	1739	1593	1106	955	936	1100	0	356
Anterior corona radiata (L)	1846	1774	680	1239	1111	1283	0	331
Superior corona radiata (R)	1369	996	177	712	440	717	0	86
Superior corona radiata (L)	1346	1182	99	709	263	390	0	56
Posterior corona radiata (R)	790	526	23	509	234	247	0	5
Posterior corona radiata (L)	715	474	0	333	191	151	0	0
Posterior thalamic radiation (R)	1168	1124	0	622	229	473	0	0
Posterior thalamic radiation (L)	1019	966	0	299	423	375	0	0
Sagital stratum (R)	640	626	0	319	0	91	0	0
Sagital stratum (L)	526	469	0	286	37	174	0	0
External capsule (R)	815	351	31	469	0	158	0	0
External capsule (L)	880	471	0	447	264	273	0	0
Cingulate gyrus (R)	361	250	0	0	0	3	0	1
Cingulate gyrus (L)	396	367	0	107	13	1	0	0
Cingulate hippocampal (R)	247	0	0	108	0	61	0	0
Cingulate hippocampal (L)	210	0	0	0	32	60	0	1
Fornix/stria terminalis (R)	283	218	0	187	0	33	0	0
Fornix/stria terminalis (L)	319	61	0	282	80	111	0	0
Superior longitudinal fasciculus (R)	1537	1408	70	1025	113	188	0	0
Superior longitudinal fasciculus (L)	1449	1181	0	848	224	170	0	0
Superior fronto-occipital fasciculus (R)	64	57	0	56	0	37	0	0
Superior fronto-occipital fasciculus (L)	58	58	0	55	0	35	0	0
Inferior fronto-occipital fasciculus (R)	522	220	4	464	0	201	0	1
Inferior fronto-occipital fasciculus (L)	520	408	0	369	177	181	0	0
Uncinate fasciculus (R)	60	22	0	59	0	38	0	0
Uncinate fasciculus (L)	46	20	0	0	0	0	0	1
Tapatum (R)	23	14	0	23	2	2	0	0
Tapatum (L)	4	0	0	3	3	3	0	0

Abbreviations: L, left; R, right.
Columns A–G include voxels that met statistical significance in their relationships to specific variables after correction for multiple comparisons.
(A) Negative correlation between FA and age.
(B) FA(HC) > FA(SP).
(C) Positive correlation between FA(SP) and positive PANSS scores.
(D) Interaction between FA and MATRICS overall score across both groups.
(E) Positive correlation between FA and MATRICS overall score in HC group.
(F) Negative correlation between FA and MATRICS overall score in SP group.
(G) Intersection of regions specified by conditions B, C, and E.
See Supplementary Table for demographic and clinical characteristics of the study sample.

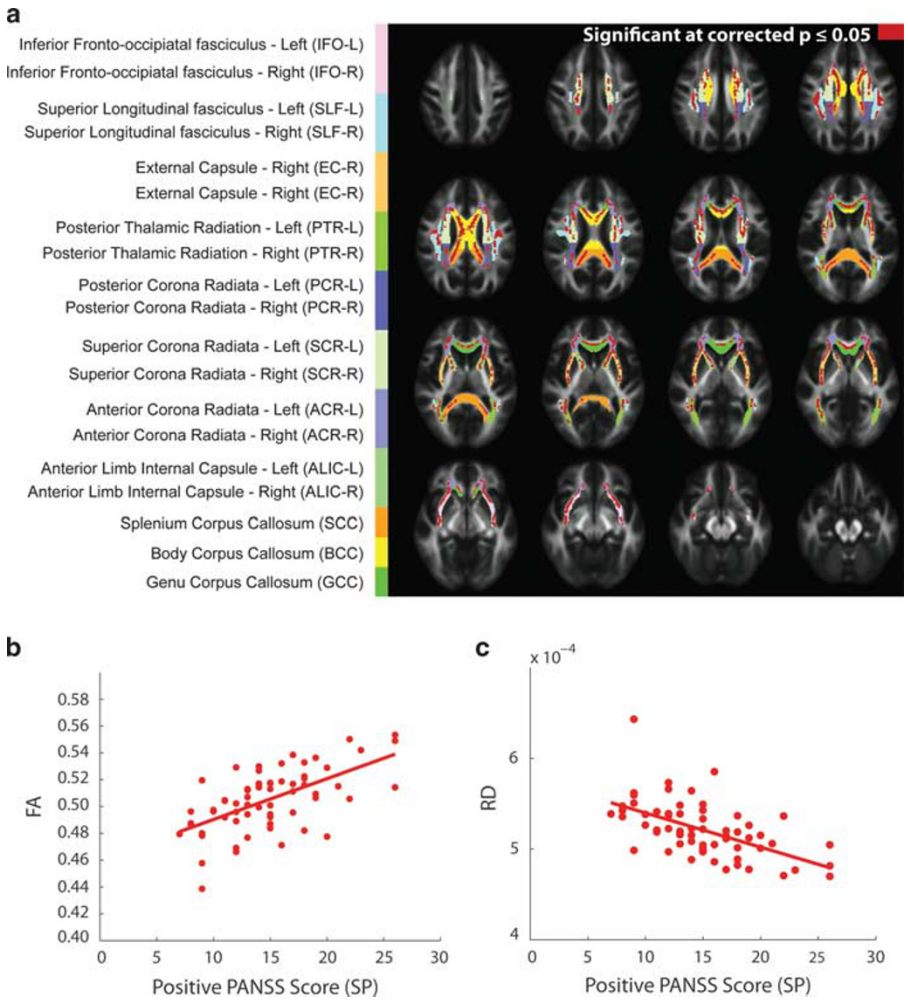

Figure 2 (a) Spatial distribution of voxels (in red) with a significant relationship between FA and positive symptoms in schizophrenia. (b) Scatter plot of mean FA (from 2a) *vs* positive symptoms in schizophrenia group ($r_{62} = 0.59$, $p < 0.0001$; $n = 64$). (c) Scatter plot of the mean RD values of voxels with a significant correlation with positive symptoms in the schizophrenia group ($r_{63} = 0.57$, $p < 0.0001$; $N = 64$).

of significant interaction, an analysis for mean FA showed a positive correlation for HCs ($r_{62} = 0.54$, $p < 0.0001$) and a negative correlation for SPs ($r_{62} = -0.27$, $p = 0.03$; Figure 3b). Similarly, in regions with MATRICS and group interaction for RD, there was a corresponding negative RD relationship with MATRICS for HCs ($r_{62} = -0.51$, $p < 0.0001$) and a positive correlation for SPs ($r_{62} = 0.28$, $p = 0.03$; Figure 3c). Because of the presence of interaction, separate analyses for each group demonstrated a positive correlation between FA and MATRICS scores for the HCs, but no relationships in the SPs, across many voxels after multiple comparison correction; the Table 1 (column E) shows the spatial distribution of the significant voxels for HCs. A corresponding pattern was identified for RD, but not for AD.

Next, in order to further understand the relationship between FA and cognition in SP, we explored regions where: (i) the MATRICS by group interaction (α_7) was significant; and (ii) FA was not significantly correlated with MATRICS scores in the HCs. These regions more directly test a negative correlation between FA and MATRICS scores in SPs. After restricting the mask for multiple comparisons to the regions defined by conditions (i) and (ii), we found a significant negative correlation only in the body of the corpus callosum ($r_{62} = -0.42$, $p = 0.0005$); the Table 1 (column F) provides the distribution of these voxels.

To examine if the same regions would exhibit the principal findings described above, we tested voxels where: (i) FA was lower in SPs; and (ii) FA was correlated with positive PANSS scores; and (iii) FA correlated with MATRICS scores in HCs. The Table 1 (column G) provides their spatial distribution. These voxels are primarily in the genu and the body of the corpus callosum, and both sides of the anterior and the superior corona radiata.

Finally, adjusting for psychotropic exposure, history of cannabis/stimulant use, or vascular risks did not significantly affect the FA and RD group differences, or their relationships with cognition across groups, or the correlations with symptoms in the SP group (all p-values < 0.05).

NAAc Differences and Correlations

SPs had a greater reduction of NAAc with age than HCs ($F_{1,121} = 4.31$, $p = 0.04$). However, the NAAc difference between the oldest (median split > 36 years) SP and HC

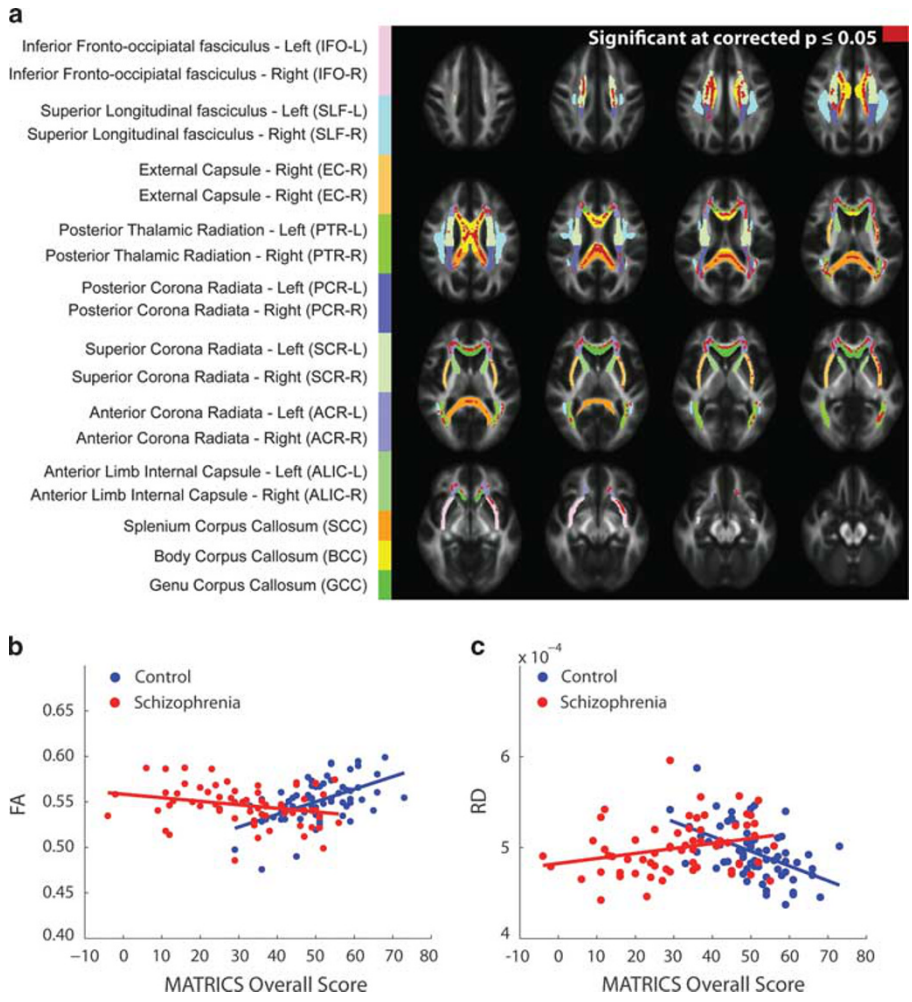

Figure 3 (a) Spatial distribution of voxels (in red) with a significant interaction between MATRICS total score and group (schizophrenia and control subjects) as these relate to FA. (b) Scatter plot of mean FA *vs* MATRICS total score (from a) in the healthy control ($r_{62} = 0.54$, $p < 0.0001$; $n = 64$) and the schizophrenia ($r_{62} = -0.27$, $p = 0.03$; $n = 64$) groups. (c) Scatter plot of the mean RD values of voxels with a significant interaction between MATRICS total score and group in the healthy control ($r_{62} = -0.51$, $p < 0.0001$; $n = 64$) and the schizophrenia ($r_{62} = 0.28$, $p = 0.03$; $n = 64$) groups.

subgroups was not significant ($F_{1,59} = 1.94$, $p = 0.17$). Controlling for age, negative symptoms positively correlated with global NAAc ($F_{1,60} = 51.9$, $p < 0.0001$). This relationship was apparent in both WM frontal regions (left: $F_{1,62} = 31.7$, $p < 0.0001$ and right: $F_{1,62} = 10.7$, $p = 0.002$) and in parietal regions (left: $F_{1,62} = 11.4$, $p = 0.001$ and right: $F_{1,62} = 4.5$, $p = 0.04$). Controlling for age, positive symptoms negatively correlated with NAAc ($F_{1,60} = 34.3$, $p < 0.0001$). This relationship was apparent in both WM frontal regions (left: $F_{1,61} = 24.0$, $p < 0.0001$ and right: $F_{1,61} = 24.5$, $p < 0.0001$) and in right parietal ($F_{1,62} = 12.9$, $p = 0.001$) but not left ($F_{1,121} = 2.2$, $p = 0.15$) regions. There were no relationships between NAAc and OLZ ($F_{1,60} = 1.2$, $p = 0.28$).

Group interacted with MATRICS score and age ($F_{2,113} = 3.6$, $p = 0.03$). However, regardless of age, HCs had the expected positive relationship between NAAc and MATRICS (Deary *et al*, 2006); Schmithorst *et al*, 2005) whereas SP had a negative relationship ($F_{1,113} = 72.8$, $p < 0.0001$; Figure 4). This relationship was apparent in both WM parietal regions (left: $F_{1,121} = 23.3$, $p < 0.0001$ and right: $F_{1,121} = 38.7$, $p < 0.0001$) and in the right frontal ($F_{1,121} = 8.9$, $p = 0.003$) but not the left frontal ($F_{1,121} = 0.56$, $p = 0.46$) regions. In addition, MATRICS was negatively correlated with negative symptoms in the SPs ($r_{63} = -0.34$, $p = 0.007$), but not with positive symptoms ($r_{63} = 0.13$, $p = 0.30$). Negative and positive symptoms were not correlated ($r_{63} = 0.06$, $p = 0.64$). Finally, adjusting for psychotropic exposure, history of cannabis/stimulant use or vascular risks did not significantly change the relationships between NAAc and age or cognition across groups or the correlations with symptoms in the SP group (all p-values < 0.05, except for the group by age interaction with vascular risk, $p = 0.06$, and with stimulant use, $p = 0.17$).

DISCUSSION

Our results are indeed paradoxical. In SP we confirmed FA reductions in several WM regions, remarkably similar to the ones described in a recent large study with TBSS (Skudlarski *et al*, 2013; however we did not find occipital reductions). Greater reduction of WM NAAc with age in SP was

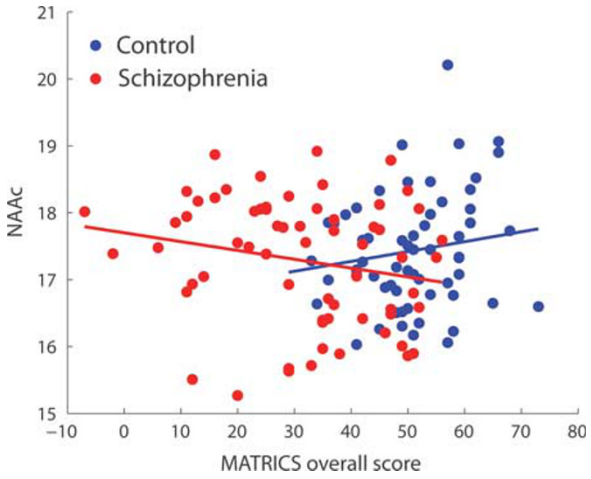

Figure 4 Scatter plot of mean white matter NAAc *vs* MATRICS total score in the healthy control ($r_{60}=0.22$, $p=0.09$; $n=61$) and the schizophrenia ($r_{63}=-0.31$, $p=0.01$; $n=64$) groups.

additionally detected, consistent with our previous study (Bustillo *et al*, 2011). However, we also found that these two measures of WM physiology, for the most part correlated counter-intuitively with the core symptom domains of SP: the higher the FA, the greater the positive symptoms, and the higher NAAc, the worse negative symptoms and cognitive function. Only positive symptom severity correlated negatively with NAAc. The spatial distribution of the FA/psychosis association was remarkable because it involved most of the analyzed FA skeleton (47 of 50 regions, though not most voxels in these regions). The NAAc/cognition relationship was present bilaterally in parietal WM, as well as in the right frontal region. Finally, both FA and NAAc had the expected positive correlations with cognition in HCs (Deary *et al*, 2006), supporting the validity of these measurements.

Several studies have reported positive associations between psychotic symptoms and FA in SP using region-of-interest, voxel-based (VB), TBSS and tractography approaches, with samples between 10 (Mulert *et al*, 2012) and 34 (Cheung *et al*, 2011). The majority of them also reported reduced FA (Choi *et al*, 2011; Hubl *et al*, 2004; Lee *et al*, 2013; Seok *et al*, 2007; Shergill *et al*, 2007; Szeszko *et al*, 2012; Whitford *et al*, 2010). The positive correlations with psychotic symptoms have been reported in several structures including left hemisphere (Hubl *et al*, 2004), left (Seok *et al*, 2007) and bilateral superior longitudinal fasciculus (Shergill *et al*, 2007), left inferior fronto-occipital fasciculus (Szeszko *et al*, 2008), anterior commissure (Choi *et al*, 2011), corpus callosum (Mulert *et al*, 2012; Whitford *et al*, 2010), and other regions (Cheung *et al*, 2011; Lee *et al*, 2013). However, others have reported negative correlations between FA and psychotic symptoms (Kitis *et al*, 2012; Skelly *et al*, 2008); (Boos *et al*, 2013; Catani *et al*, 2011; Cui *et al*, 2011). An important negative finding with VB is the study in mostly drug-naive first episode patients (SP, $N=122$; Wang *et al*, 2013). Finally, most ^{1}H-MRS studies have not reported significant NAAc correlations with symptoms (Kraguljac *et al*, 2012). However, some have found a negative relationship between WM NAAc with positive and with negative symptoms (He *et al*, 2012).

Our study is generally consistent with the majority of this literature in chronically ill patients. However, the results are striking for the much broader spatial distribution of the FA/positive symptom relationship. This included several of the regions previously reported as potentially representative of networks involving inner speech (eg: superior longitudinal, inferior fronto-occipital fasciculi), as well as others not typically thought to be involved in language (eg: corticospinal tract, cerebral peduncles; see the Table 1, column B). Consistent with the previous literature in SPs, none of the regions that had this relationship were higher in FA than HCs.

Differences in sample characteristics and DTI and ^{1}H-MRSI methodology may account for differences with the existing literature. Our sample was larger than most previous studies, except for two. One (Boos *et al*, 2013) utilized tractography, acquired at 1.5 T and found no FA reductions. Perhaps the averaging of FA along each tract, reduced the sensitivity in this study to detect the expected FA reduction in SP, as well as positive relationships with psychotic symptoms. The other (Wang *et al*, 2013) studied mostly drug-naive patients with VB. Our subjects were chronically ill but clinically stable, perhaps exhibiting more trait-like positive symptoms. Regarding methods, we used higher field strength providing greater signal to noise (predominantly in the periphery) than most previous studies. Also, use of TBSS provided broad unbiased spatial coverage with reduced partial volume effect (Smith *et al*, 2006), a limitation of VB.

Three studies have acquired DTI and ^{1}H-MRS (single-voxel) in schizophrenia, in chronic patients and with smaller samples. Two studies (Tang *et al*, 2007; Steel *et al*, 2001) reported decreased WM NAAc. Tang *et al*, (2007), and Rowland *et al*, (2009) found reduced FA. None found relationships with symptoms or cognition. Finally, though the metanalysis found reduced NAAc in several WM regions (Steen *et al*, 2005), differences in the centrum semiovale were smaller, somewhat consistent with our negative findings.

The broadly distributed positive correlations between FA and cognition found in the HCs are consistent with previous literature (Deary *et al*, 2006; Schmithorst *et al*, 2005). This pattern was clearly lost in the schizophrenia group and even followed an inverse correlation in the corpus callosum, suggesting that, as with positive symptoms, FA values closer to the normal range correspond to greater pathology. The reported relationship between FA and cognition in schizophrenia has tended to be positive mainly early in the illness (Wang *et al*, 2013; Perez-Iglesias *et al*, 2010). However, our ^{1}H-MRSI results clearly converge with the FA findings: NAAc correlated positively in HC but negatively in the schizophrenia group, supporting the validity of results in this sample.

What is the meaning of this counter-intuitive relationship of FA increasing with symptoms? FA is a composite measure related to myelination and axonal coherence, including both normal alignment and complexity (number of crossing fibers; however, the relationship between FA and myelin has been questioned; Sen and Basser, 2005). In WM, NAAc is found almost exclusively in axons and its concentration indicates axonal density (Rae, 2014). In addition, schizophrenia is a heterogeneous disorder and different aspects and stages of the illness may affect FA and NAAc through separate and even opposing mechanisms. Furthermore, the effect of antipsychotic medication cannot be discounted (Wang *et al*, 2013; Reis Marques *et al*, 2014). A trait

dysfunction in myelination may account for the primary reduction in FA in schizophrenia (as supported by post-mortem and genetic studies (Hoistad *et al*, 2009), but also by lower FA in unaffected family members (Skudlarski *et al*, 2013). However, we did not find RD group differences. Still, in the more psychotic patients, we see increased structural connectivity (higher FA) perhaps secondary to increased myelination (lower RD), with lower axonal density (reduced NAAc). We speculate that the underlying tissue changes may involve increased oligodendrocytes, which would account for both increased myelin and reduced axonal density. Regarding the chronology of presentation of the relationships between FA and psychosis in schizophrenia, we posit two alternative models.

In an Adaptive framework (model-1), chronically psychotic subjects may develop increased myelination across various tracts, like those involved in the generation and monitoring of inner speech (Shergill *et al*, 2007), but eventually distributed though-out many WM regions. Our findings involving bilateral regions like internal and external capsule, corona radiata and superior and inferior longitudinal fasciculi (Table 1 column C) are consistent with bilateral fronto-temporal cortical regions of increased functional connectivity in schizophrenia patients with hallucinations (Jardri *et al*, 2011).

Alternatively, in a Cohort framework (model-2), patients with already increased myelination in critical networks may be predisposed to more sustained positive symptoms. However, contrary to model-2, the largest study of drug-naive patients (Wang *et al*, 2013) found reduced FA but no relationship with positive symptoms.

SPs had absent relationships of FA with cognition and in parts of the corpus callosum there was a negative correlation. With NAAc, the relationships were clearly negative. Though cognition is normally supported by higher FA (perhaps because of optimal myelination and axonal coherence) and higher NAAc, in schizophrenia it may be further affected by other neurobiological variables which may lead to compensatory remodeling of WM tracts to support cognition later in the illness (model-1). We speculate of a selective reduction of the more aligned axons, leaving fewer (reduced NAAc) but mainly crossing fibers (reduced FA), in those more cognitively intact SPs. Regardless of mechanism, the positive correlation between FA and cognition found early in the illness in the largest sample to date (Wang *et al*, 2013), argues against model-2 and suggests an undetermined adaptive process resulting in a fundamental microstructural reorganization of WM latter in the illness.

The diffusion, relaxation, and neurochemical characteristics of WM in schizophrenia are complex. In chronically ill medicated patients, both increased water T_2, as well as decreased NAA T_2 were found (Du *et al*, 2012) suggestive of reduced macromolecules and abnormal intra-axonal milieu, respectively. A follow-up study reported decreased magnetization transfer, consistent with reduced myelin, but increased NAAc diffusion. Though both of these abnormalities could lead to reductions in FA, they did not correlate with each other, suggesting 'independent mechanisms leading to myelin and axonal abnormalities' (Du *et al*, 2013). In addition NAAc diffusion and T_2 relaxation were not related (Du *et al*, 2013), further arguing for the complexity of WM microstructural changes in schizophrenia.

This study had several strengths, including unbiased assessment of WM with automated DTI and ^{1}H-MRSI analytic tools, contrasts of FA, RD and AD, reliable symptom assessments, standardized cognitive battery and a large sample. However, limitations should be acknowledged. First, patients were chronically ill and treated with antipsychotic medications, which have been reported to increase (Reis Marques *et al*, 2014) and decrease (Wang *et al*, 2013) FA and perhaps confound the relationships we found. However, current antipsychotic dose did not account for our results. Long-term treatment studies assessing antipsychotic compliance in different stages of the illness are necessary. Second, TBSS does not measure FA along tracts associated with specific brain function. Although some tractography-based studies have found similar relationships with psychosis (Lee *et al*, 2013), others have not (Boos *et al*, 2013). Third, our DTI sequence was not cardiac-gated. Fourth, we did not assess length of substance-use history. Fifth, diffusion and NAAc measures were not spatially co-localized. Finally, the cross-sectional study design supports mainly the descriptive and not the causal interpretations.

In summary we report, in the context of broadly reduced FA but normal WM NAAc in SP, positive correlations between positive symptoms and FA, but negative ones between these symptoms and NAAc and RD, suggestive of increased myelination throughout multiple WM bundles among the most psychotic patients. A separate set of abnormal relationships between cognition and FA, as well as with NAAc, converge to suggest that a fundamentally different WM microstructure supports the two core illness domains: psychosis and cognitive/negative symptoms. In the context of the current literature, an adaptive process evolving later in the illness is most consistent with these findings. Future longitudinal studies examining the evolution of these relationships and their specificity to myelination, axonal coherence, and functional connectivity may shed light on the underlying neurobiology of persistent psychosis and cognitive deficits.

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The authors declare no conflict of interest.

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