Reduced fronto-temporal connectivity is associated with frontal gray matter density reduction and neuropsychological deficit in schizophrenia

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A R T I C L E   I N F O

Article history:
Received 29 July 2008
Received in revised form 13 October 2008
Accepted 8 November 2008
Available online 18 December 2008

Keywords:
Schizophrenia
Diffusion tensor imaging
Voxel-based morphometry
Neuropsychology

A B S T R A C T

Objectives: A “disconnectivity model” of schizophrenia has been proposed, but it is still unclear if white matter abnormalities are associated with gray matter changes and if they may be the anatomic substrate of cognitive impairment, which is a core symptom of the disorder. The first objective was to detect if white matter microstructure alterations in schizophrenia are associated with or independent of gray matter change, using an optimized method for white matter (Tract-Based Spatial Statistics) and gray matter analyses (whole-brain voxel-wise approach). The second objective was to identify the neuropsychological correlates of white matter abnormalities in the schizophrenic group, using a comprehensive neuropsychological battery.

Methods: In this case-control study 43 schizophrenic patients and 43 healthy volunteers were consecutively enrolled and matched for age and gender.

Results: Fractional anisotropy reduction was found in 6 fronto-temporal clusters (corrected p-values < 0.05) in schizophrenic group in comparison with healthy volunteers, and 3 clusters showed fractional anisotropy increase (corrected p-values < 0.05). Two of the clusters showing reduced fractional anisotropy were associated with reduced gray matter density in neuroanatomically-related regions in schizophrenic subjects (p-values ranging from 0.001 to 0.026). Executive, constructional-praxis, and working memory deficits were significant predictors of fractional anisotropy reduction in 4 clusters in the schizophrenic group (p-values ranging from <0.0001 to 0.0017).

Conclusions: Our data support the disconnectivity hypothesis in schizophrenia, enlightening a link between reduced fronto-temporal connectivity and “frontal” cognitive deficits. Reduced gray matter density may be involved primarily in the pathogenesis of some of these disconnected areas.

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1. Introduction

Schizophrenia has been considered as a disconnection syndrome (Andreasen et al., 1998; Crow, 1998b; Davis et al., 2003; McGuire and Frith, 1996; Spalletta et al., 2003), with a deficit in white matter (WM) integrity leading to a defective communication among brain regions. This hypothesis has been supported by post-mortem studies showing abnormalities in the myelin sheath (Uranova et al., 2001) and oligodendroglia (Hof et al., 2003) and by evidence of a decreased expression of myelination-related genes in schizophrenia (Hakak et al., 2001; Jungerius et al., 2007; Karoutzou et al., 2008; McIntosh et al., 2007).

An indicator of WM integrity is Fractional Anisotropy (FA) obtained from Diffusion Tensor Imaging (DTI) (Assaf and...
Abnormal WM myelination during the development has been hypothesized to lead to aberrant cortical folding pattern, such as gyral asymmetry abnormalities, in frontal (Naritoku et al., 2004; Wisco et al., 2007) and temporal (Naritoku et al., 2004, 2001) regions in schizophrenic patients. A decrease in WM integrity in schizophrenia could be due to different mechanisms, such as a disrupted axonal myelination, or the axonal elimination resulting from neuronal death. According to the latter hypothesis, WM integrity reduction should be associated with gray matter (GM) reduction (Farrow et al., 2007; Shenton et al., 2001; Suzuki et al., 2005) and in association with cognitive impairment (i.e. executive functioning, working memory and verbal learning) (Goldman-Rakic and Selemon, 1997; Rusch et al., 2007). Similarly, a lower FA has been found with a whole brain analysis in schizophrenic patients. A decrease in WM integrity between adult schizophrenic patients and healthy comparison (HC) subjects, using the recent Tract-Based Spatial Statistics (TBSS) method, which increases the sensitivity and the interpretability of the results compared with voxel-based approaches based purely on non-linear registration (Smith et al., 2006). We expected that schizophrenic patients would demonstrate lower FA compared to HC mainly in frontal and temporal WM fibers.

Finally, we aimed to investigate the presence of neuro-psychological correlates of WM microstructure abnormalities in schizophrenic patients. Since prior DTI studies in schizophrenia have described associations between lower FA in fronto-temporal fibers and reduced working memory (Karlsgodt et al., 2008) and executive function performances (Kubicki et al., 2003; Nestor et al., 2004, 2008), we hypothesized that lower FA in frontal and temporal fiber tracts would be associated with worse executive and working memory functions.

2. Methods

2.1. Subjects

Sixty consecutive patients with a diagnosis of schizophrenia according to DSM-IV (APA, 1994) were initially recruited from two outpatient clinics in central Italy. Diagnosis of schizophrenia was independently performed by three clinicians, who had been trained until an interrater reliability level of κ≥0.80 was reached. Specifically, two clinicians (G.M., I.A.R.), who treated the patients and knew their clinical history, used DSM-IV criteria to make a preliminary diagnosis of schizophrenia. The two clinicians were blind to the prior diagnosis of the patient and to the aims of the study. All diagnoses were then confirmed or not on the day of image acquisition by one senior clinical psychiatrist (G.S.) using the structured clinical interview for DSM-IV (SCID-P) (First et al., 1997b). The senior psychiatrist also screened the patients according to the inclusion and exclusion criteria of the study. In the case of disagreement between the senior psychiatrist and the two clinicians who had made the preliminary diagnosis, more data were requested to help resolve the differences, and the diagnostic process continued until a final consensus diagnosis was assigned. If the three diagnosicians could not reach an agreement, the patient was removed from the sample.

Severity of schizophrenia was assessed by using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) (mean total PANSS score±SD=96.93±20.77; mean positive symptoms score±SD=22.26±6.23; mean negative symptoms score±SD=74.66±17.38).
score±SD=23.77±7.61; mean general psychopathology score±SD=50.91±11.70). Age at onset was defined as age at first hospitalization or, where possible, age at onset of positive or negative symptoms preceding the first hospitalization. All the patients included were between 18 and 66 years of age, were right-handed, and had completed at least 5 years of schooling.

Exclusion criteria were: history of alcohol or drug dependence or traumatic head injury; any past or present major medical or neurological illness; any additional psychiatric disorder; any brain pathology identified on T2- or FLAIR-scans; and mental retardation. All patients were receiving stable oral doses of one or more atypical antipsychotic drug such as risperidone, quetiapine, and olanzapine. Antipsychotic dosages were converted to estimated equivalent dosages of olanzapine.

Out of the 60 patients in the initial sample, 17 patients were excluded according to the above-mentioned exclusion criteria. Specifically, seven patients had history of alcohol and/or psychoactive drug dependence, two had traumatic head injury, one suffered from mental retardation, three met criteria for other psychotic disorders according to the diagnosis made by the psychiatrist who administered the SCID-P (one delusional disorder, two schizoaffective disorder), and four patients were removed from subsequent analyses due to significant movement artefacts during the MRI procedure. Thus, the final sample consisted of 43 schizophrenic patients.

Forty-three HC subjects were recruited in the same geographic area and rigorously matched with the schizophrenic patients for age and gender (Table 1). All the HC were carefully screened for a current or past diagnosis of any axis I disorder; any brain pathology identified on T2- or FLAIR-scans; and mental retardation. All the HC were rigorously matched with the schizophrenic patients for age and gender (Table 1). All the HC were carefully screened for a current or past diagnosis of any axis I disorder; any brain pathology identified on T2- or FLAIR-scans; and mental retardation. All the HC were rigorously matched with the schizo-

The study was approved and undertaken in accordance with the guidance of our local Ethics Committee and written consent was obtained from all participants after a full explanation of the procedures of the study.

2.2. Image acquisition

The 86 participants underwent the same imaging protocol with a whole-brain T1-weighted and diffusion-weighted scanning using a 3 T Allegro MR imager (Siemens, Erlangen, Germany) with a standard quadrature head coil.

Whole-brain T1-weighted images were obtained in the sagittal plane using a modified driven equilibrium Fourier transform (MDEFT) (Deichmann et al., 2004) sequence (TE/TR=2.4/7.92 ms, flip angle 15°, voxel-size 1×1×1 mm³).

Diffusion-weighted images were obtained using echo-planar imaging (SE-EPI, TE/TR=89/8500 ms, 52 axial slices, bandwidth=1860 Hz/vx, voxel size 1.5×1.5×3.0 mm³) with 12 isotropically distributed orientations for the diffusion-sensitizing gradients at a b-value of 1000 s·mm⁻² and 2 b=0 images. Image distortions induced by eddy currents and head motion in the DTI data were corrected by applying a full affine (mutual information cost function) alignment of each image to the mean diffusion weighted image. After distortion corrections, DTI data were averaged and concatenated into thirteen (1 b0+ 12 b1000) volumes. We minimized movement by stabilizing the head with cushions and tape before scanning.

Scans were assessed by a trained radiologist, and any scan with significant artefacts was repeated.

2.3. Image analysis

2.3.1. White matter preprocessing

All FA maps were generated using DTIFit within the FMRI Diffusion Toolbox (part of FSL) (Smith, 2004). Voxel-wise differences in DTI scalar indices were assessed using TBSS (also part of FSL), a recent approach which increases the sensitivity and the interpretability of the results compared with voxel-based approaches based purely on non-linear registration (Smith et al., 2006). Ventricular enlargement caused by the pathophysiological process may for instance considerably mislead the interpretation of the voxel-based results. TBSS aims to solve the problematic issues of standard voxel-wise methods via the use of a carefully tuned non-linear registration, followed by the projection of the nearest maximum FA values onto a skeleton derived from a mean FA image. This projection step aims to remove the effect of cross-subject spatial variability that remains after the non-linear registration.

2.3.2. Gray matter preprocessing

As we aimed to investigate the correlation between FA values and GM density in schizophrenic patients across the whole brain, we used a practical and well-known methodology for the automated segmentation and registration of the brain to obtain a GM tissue map for each patient. A density map reflects the voxel values of GM segments, i.e. the local presence, or concentration, of GM.

The MDEFT images were processed following an ‘optimized’ VBM protocol (Good et al., 2001) using the standard segmentation and registration tools available in the statistical parametric mapping software (SPM5, www.fil.ion.ucl.ac.uk/spm)

---

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Schizophrenic patients (N=43)</th>
<th>Comparison subjects (N=43)</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.23±11.97</td>
<td>41.30±21.46</td>
<td>0.285</td>
<td>84</td>
<td>0.776</td>
</tr>
<tr>
<td>Educational level (years)</td>
<td>11.63±3.29</td>
<td>14.46±3.14</td>
<td>4.091</td>
<td>84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at the onset of the illness (years)</td>
<td>24.14±8.90</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Duration of the illness (years)</td>
<td>16.09±12.28</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olanzapine Equivalents (mg/day)</td>
<td>20.95±34.44</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gender (male) DSM-IV subtypes: Paranoid schizophrenia</td>
<td>24 (56)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Paranoid schizophrenia</td>
<td>3 (7)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Disorganized schizophrenia</td>
<td>8 (18.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Undifferentiated schizophrenia</td>
<td>8 (18.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Residual schizophrenia</td>
<td>8 (18.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

SD = standard deviation; df = degrees of freedom.
To optimize brain extraction and tissue segmentation, each Magnetic Resonance Image (MRI) brain density map was positioned along the anterior–posterior commissure line and was rotated so that the septum pellucidum and at least a large part of the falk would be visible in the sagittal plane. Then the unified segmentation step in SPM5 was run. This function in SPM5 can be used for bias correction, spatial normalization (using a combination of linear and non-linear functions), and segmentation of the images in GM and WM partitions and in cerebrospinal fluid (Ashburner and Friston, 2005).

Finally, all GM images were smoothed with a 12 mm$^3$ FWHM Gaussian kernel. Statistical analysis was performed only on the GM tissue images.

### 2.4. Cognitive examination

Two trained neuropsychologists (I.S. and G.B.), who were blind to the aims of the study, conducted the cognitive assessment, which was performed within fifteen days of MRI. Acceptable interrater reliability level for the present study was defined as of $k > 0.80$. Thirty-seven patients completed the entire neuropsychological battery and 6 refused.

To obtain a global index of cognitive impairment, the Mini Mental State Examination (MMSE) was used (Folstein et al., 1975). Furthermore, we selected the following tests from the Mental Deterioration Battery (MDB) (Carlesimo et al., 1996) in order to provide information about the functionality of different cognitive domains such as: verbal memory (MDB Rey's 15-word Immediate Recall (RIR) and Delayed Recall (RRD)); short term visual memory (MDB Immediate Visual Memory (IVM)); logical reasoning (MDB Raven's Progressive Matrices' 47 (PM47)); language (MDB Phonological Verbal Fluency (PVF), and MDB Sentence Construction (SC)). We also added the Category Fluency (CF) test (Lucas et al., 1998) in order to assess semantic fluency ability.

As “executive functioning” denotes a set of different cognitive abilities which are involved in complex, goal-directed thought and behaviour (Kerns et al., 2008), the following executive dimensions were assessed: a) attentional control; b) set-shifting; c) perceptual organization and planning; d) working memory.

In order to assess abilities of attentional control and inhibition we administered the Stroop test (ST) (Stroop, 1935). The ST consists of three parts: in the ‘word reading’ task, participants were asked to read as quickly as possible words indicating colours (Italian words for ‘blue’, ‘red’, ‘green’, and ‘yellow’) that were printed in black ink on a white sheet. For the second part (‘colour naming’), participants were shown a sequence of blue, red, green, and yellow dots and asked to name the colours as quickly as possible. The third part of the test (‘interference condition’) consisted of words indicating colours but printed with a coloured ink not correspondent to the colour word (e.g., the word ‘blue’ printed in red ink). Participants were requested to name the ink colour of the word, as quickly as possible (in the example, to say “red”). In the case of a reading error the patient was stopped and ordered to go back to the previous word. Time of performance during the interference subtest was chosen as measure.

Set-shifting or cognitive flexibility, that is the ability to alter a behavioural response mode in the face of changing contingencies, was assessed using the Modified Wisconsin Card Sorting test (MW CST) (Heaton et al., 1993). In this test, subjects were asked to sort cards according to a criterion (colour, number, or form) and then to shift to a new criterion following the feedback of the examiner. The number of completed categories and the number of perseverative errors were chosen as measures.

We also administered the Rey–Osterrieth complex picture test. It is a measure of visual perception and constructional praxis (Osterrieth, 1944; Rey, 1941) which also provides assessment of a number of other cognitive processes including perceptual organizational skills, executive function (i.e. planning), and problem-solving strategies (Knight and Kaplan, 2003). For these reasons, the Rey–Osterrieth complex picture test is considered to be a useful tool for the evaluation of frontal lobe function, and has been used to identify organization/planning deficits in patients with frontal lobe lesions (Pillon, 1981). Different versions of this test exist. We administered two test conditions: the copy of Rey–Osterrieth complex picture (CROP) and its delayed recall (ROPR). In the first condition (CROP), subjects were given the stimulus card, and then asked to draw the same figure. Participants were asked to copy the figure on the sheet of paper, as carefully as they could. Subjects were not allowed to erase or to change page orientation. The ROCF was scored according to the Taylor criteria, with scores ranging from 0 to 36 (Spreen and Strauss, 1991). In the second condition (ROCRF), starting after 10 min in which only non-visuospatial trials must be administered, participants were asked to draw what they remembered, providing information about long-term visuospatial memory.

In order to measure verbal, spatial, and visual working memory we administered the “n-back” tasks (Braver et al., 1997). The n-back test has been widely used in previous research (Oliveri et al., 2001; Owen et al., 1998; Smith and Jonides, 1997; Spalletta et al., 2008).

In this test subjects are required to monitor continuously a sequence of verbal/spatial/visual stimuli (a total of twenty-two items for each task, visually presented on a screen) and to select items that appeared n-items back in any sequence (this sequence was randomly generated using locally written software installed on a Pentium© 4 IBM computer). In the verbal task, stimuli consist of a series of words; in the spatial task there is a white box which is differentially located among black boxes; finally, in the visual task, stimuli are a series of abstract pictures.

The item selection was done by the participants using a keyboard with three keys, one for each stimulus. Also, the computer software automatically generated a file with results of the task with corrected–uncorrected responses. We administered the n-back subtasks (i.e. verbal/spatial/visual) at three different levels of difficulty. At the “n-1 level” subjects were required to select an item that appeared one item back in a sequence, at the “n-2 level” to select an item that appeared two items back in a sequence, and at the “n-3 level” to select an item that appeared three items back in a sequence. The number of correct responses (“accuracy score”) was generally considered as index of working memory performance. All participants were trained to obtain their maximal performance score, using the n-1 back paradigm by means of verbal and written explanations. Training consisted in having patients practice the n-1 back paradigm three times before starting the experimental procedure. Accuracy scores as memory load at the n-2 level for
the three subtasks were chosen as measures in the statistical analyses, as patients scored at chance for the n-3 task and at ceiling for the n-1 task.

2.5. Statistical analyses

Comparisons between the two groups on sociodemographic characteristics (i.e. age and educational level) were performed using t-tests. Comparison between the two groups on gender variable was performed using the chi-square test.

2.5.1. WM comparisons between schizophrenia and HC groups

To achieve accurate inference including full correction for multiple comparisons over space, we used permutation-based non-parametric inference within the framework of the general linear model (Nichols and Holmes, 2002) to investigate changes in the distribution of FA between both groups (5000 permutations). Results were all considered significant for \( p < 0.05 \), corrected for multiple comparisons at the whole brain voxel level.

Regions of interest (ROI) for subsequent correlation analysis were obtained considering the clusters showing significant changes in FA between patients and HC. The mean FA value from these clusters was extracted from each patient, to be used in the subsequent correlation analysis with whole-brain GM.

2.5.2. Correlation between WM clusters and GM tissue density map

We performed single regression analyses to test for any correlation between the mean FA value and the GM map density in schizophrenic patient group.

Each regression model included all patients’ GM maps density and each WM cluster that showed a significant reduction or increase in patients compared to HC in the previous statistical analysis.

The statistical threshold was set to Family Wise Error (FWE) \( p < 0.05 \), corrected for multiple comparisons. We looked at the whole GM map. No ROI was selected. The stereotaxic coordinates are reported in Montreal Neurological Institute (MNI) space (Cocosco et al., 1997).

2.5.3. Correlation between WM clusters and neuropsychological scores

The neuropsychological predictors of FA value of each WM cluster in the schizophrenic subset of 37 patients were assessed by using a series of stepwise multiple regression analyses, with a forward procedure and an F to enter of 4. Pre-selection of variables to include in the stepwise regression models was performed by using correlation analyses (Pearson’s r) and Fisher’s r to z transformation in order to determine the significance of correlations for continuous variables. In the stepwise multivariate models only variables with \( p < 0.05 \) in the pre-selection analyses were included. All tests were two-tailed, and the level of statistical significance was defined as an alpha less than 0.05.

3. Results

From the 60 patients of the initial sample, 17 schizophrenic patients were excluded following the exclusion criteria (see the Methods section). The final sample of 43 schizophrenic patients and the sample of 43 HC did not differ significantly in age and gender, whereas they did differ in educational attainment (Table 1).

3.1. White matter results

TBSS mapping of anisotropy differences between schizophrenic patients and HC demonstrated a significant decrease of FA in the right hemisphere of patients in the Inferior Longitudinal Fasciculus (ILF), in the Superior Longitudinal Fasciculus subcomponent III (SLF III), and in the Uncinate Fasciculus (UF); in the left hemisphere of patients the decrease of FA was confined to the Extreme Capsule (EmC) (see Table 2 for details and coordinates).

We found a relative increase in FA of patients in Internal Capsule, posterior limb (ICp) bilaterally (see Table 3 for details and coordinates; Fig. 1).

3.1.1. Correlation between WM clusters and GM tissue maps

We found that 2 out of 6 WM clusters of decreased FA of patients correlated positively with GM tissue map density (i.e. FA value in a given WM cluster correlated with GM density in neuroanatomically related regions) (see Table 4 for details and coordinates). In particular, the FA value in cluster 7, identified as right SLF III, correlated with GM density in the Right Middle Frontal Gyrus (BA 6), Right Pars Triangularis (BA 45) and Left Middle Frontal Gyrus (BA 6).

Cluster 6, identified as right UF, correlated with Right Anterior Cingulum (BA 32) and Right Cerebellum.

None of the increased FA clusters showed correlations with GM tissue maps (Fig. 2).

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM clusters showing reduced FA in the schizophrenic group (( N = 43 )) in comparison with comparison subjects group (( N = 43 ))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Brain gyrus</th>
<th>White matter fiber</th>
<th>Side</th>
<th>Coordinates MNI</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>ITG</td>
<td>ILF</td>
<td>Right</td>
<td>44 - 36 - 11</td>
<td>286</td>
</tr>
<tr>
<td>8</td>
<td>ITG</td>
<td>ILF</td>
<td>Right</td>
<td>36 - 65 - 10</td>
<td>122</td>
</tr>
<tr>
<td>7</td>
<td>SFG</td>
<td>SLF III</td>
<td>Right</td>
<td>41 19 21</td>
<td>107</td>
</tr>
<tr>
<td>6</td>
<td>IFG</td>
<td>UF</td>
<td>Right</td>
<td>24 21 - 6</td>
<td>103</td>
</tr>
<tr>
<td>5</td>
<td>SFG</td>
<td>SLF III</td>
<td>Right</td>
<td>49 28 17</td>
<td>106</td>
</tr>
<tr>
<td>4</td>
<td>SFG</td>
<td>EmC</td>
<td>Left</td>
<td>-21 50 16</td>
<td>85</td>
</tr>
</tbody>
</table>

Cluster p-value corrected < 0.05.

ITG: Inferior Temporal Gyrus; SFG: Superior Frontal Gyrus; IFG: Inferior Frontal Gyrus; ILF: Inferior Longitudinal Fasciculus; SLF III: Superior Longitudinal Fasciculus subcomponent III; UF: Uncinate Fasciculus; EmC: Extreme Capsula.

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM clusters showing increased FA in schizophrenic patients (( N = 43 )) in comparison with control subjects (( N = 43 ))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cluster</th>
<th>White matter</th>
<th>Side</th>
<th>Coordinates MNI</th>
<th>Cluster size</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>ICp</td>
<td>Right</td>
<td>15 0 4</td>
<td>286</td>
</tr>
<tr>
<td>2</td>
<td>ICp</td>
<td>Left</td>
<td>-12 -2 0</td>
<td>122</td>
</tr>
<tr>
<td>1</td>
<td>ICp</td>
<td>Left</td>
<td>-26 -13 -4</td>
<td>109</td>
</tr>
</tbody>
</table>

Cluster p-value corrected < 0.05.

ICp: Internal Capsule, posterior limb.
3.1.2. Cognitive predictors of WM abnormalities in schizophrenia

Thirty-seven patients completed the entire neuropsychological assessment. This subset did not differ from the whole patient sample for age (subset of patients: mean±S.D.=41.8±11.71; whole patient sample: 40.2±11.97), education (subset of patients: mean±S.D.=11.7±3.22; whole patient sample: 11.6±3.29), gender (subset of patients: male=22 (60%); whole patient sample: male=27 (63%)), illness duration (subset of patients: mean±S.D.=16.8±12.56; whole patient sample: 16.1±12.28), and neuroleptic treatment dosages measured as equivalents of olanzapine (subset of patients: mean ±S.D. =21.2 ±36.97; whole patient sample: 20.9±34.44).

In order to clarify the relationship between neuropsychological deficit and WM abnormality in the schizophrenic subset of 37 patients, we performed a series of stepwise multiple regression analyses with each WM cluster as dependent variable. The pre-selected neuropsychological variables that in the correlation analysis were related \((p<0.05)\) with the dependent variable (i.e. WM cluster) were included as independent variables. Illness duration was included in all the stepwise multiple regression analyses as independent variable, in order to exclude a confounding role of this factor on WM microstructure integrity.

The analyses showed that significant predictor of cluster 4 (i.e. left EmC) FA score was CROP score. The resulting equation was significant \((F=11.577; \text{df}=1.35; p=0.0017)\) and explained 24.9\% \((r^2)\) of the overall variance of cluster 4 FA score. In particular, lower CROP scores predicted the lower FA values in the left EmC.

Significant predictors of cluster 6 FA values, corresponding to the right UF, were CROP scores and the number of completed categories at MWCST. The resulting equation was significant \((F=12.722; \text{df}=2.34; p<0.0001)\) and explained 42.8\% \((r^2)\) of the overall variance of cluster 6 FA values. In particular, lower CROP scores and lower number of completed categories at MWCST predicted the lower FA values in the right UF.

Significant predictors of cluster 9 (i.e. right ILF) FA values were CROP and n-back verbal n-2 task scores. The
resulting equation was significant ($F=13.455; \text{df}=2.34; p<0.0001$) and explained 44.2% ($r^2$) of the overall variance of cluster 7 FA values. In particular, lower CROP scores and lower n-back verbal n-2 task scores predicted the lower FA values in the right SLF III.

None of the increased FA clusters was predicted by neuropsychological performance.

4. Discussion

TBSS analysis revealed that patients with schizophrenia in comparison to HC subjects are characterized by decreased WM integrity within several fiber tracts linked to frontal and/or temporal cortices and most of them right-lateralized. Furthermore, in the schizophrenic sample, 2 out of the 6 WM clusters of decreased FA are associated with a reduced GM density in related regions. Finally, specific neuropsychological deficits are significant predictors of reduced WM microstructure integrity in the subset of 37 patients which completed the cognitive assessment.

4.1. White matter abnormalities in schizophrenia and related cognitive deficits

Our findings of a reduced FA in the right ILF, the right SLF III, the right UF, and the left EmC indicate a decreased connectivity between brain regions linked to frontal and temporal circuits. These results are consistent with evidence from other DTI (Jones et al., 2006; Kanaan et al., 2005; Kubicki et al., 2005, 2002; Seal et al., 2008; Skelly et al., 2008), histological (Benes et al., 1986, 2001; Bouras et al., 2001), and functional studies (Carter et al., 2001, 1998; Cohen et al., 1998; Nordahl et al., 2001), supporting the hypothesis of an inefficient communication between frontal and temporal lobes in schizophrenia (Winterer et al., 2003). Furthermore, we found significant cognitive predictors of almost all the WM clusters showing reduced FA. Particularly, a complex constructive-praxis task (CROP) is significant predictor of all the reduced FA clusters, whereas verbal working memory deficit, measured by verbal n-2 task (n-back test), is predictor of FA reduction in SLF III, and the executive ability of set-shifting, as assessed by MWCST, predicts FA values in ILF and UF. We explain these results considering the anatomical connections of these fiber tracts and their functional meaning.

The EmC divides into a superior ramus that lies in the WM of the inferior frontal lobe, and an inferior ramus that lies beneath the claustrum on the floor of the orbital cortex, laterally adjacent to the fibers of the UF. Considering EmC's connections, it has been hypothesized (Crosby and Section, 1982) that the rostral prefrontal cortex exerts its control, via the EmC, on the most highly integrated cortical information, and its disconnections may be the anatomical basis for an impairment in abstract information processing (Christoff et al., 2003) and in the integration of multiple cognitive operations (Petrides, 2005; Ramnani and Owen, 2004) in schizophrenia. We found that significant predictor of EmC FA values is CROP score. Indeed, the Rey–Osterrieth Picture is an abstract and complex stimulus, whose copy requires strategic planning and organizing abilities (Pillon, 1981). Specifically, reproducing the Rey–Osterrieth Picture involves the ability to organize the figure into a meaningful perceptual unit (Pillon, 1981) and to integrate different cognitive information, a multiple cognitive process which could be underpinned by EmC's connections.

Similarly, the right ILF showed a reduced FA in our schizophrenic sample compared to HC sample, consistently with several DTI studies (Buchsbaum et al., 1998; Lim et al., 1999; Minami et al., 2003; Skelly et al., 2008). The ILF links the ventral and lateral occipital regions with the ventral temporal cortices, which are important for object recognition, and the posterior parahippocampal gyrus, which is engaged in memory and discrimination tasks (Squire and Zola-Morgan, 1991; Ungelerde and Mishkin, 1982). We found that significant predictors of FA value in right ILF are CROP score and number of completed MWCST categories, which tests are both composed of visual stimuli involving discrimination and object recognition abilities. Thus, a decrease in WM connectivity in this fiber tract may explain these neuropsychological deficits.

TBSS analysis also revealed a reduced FA in two other fiber tracts linked to the frontal cortex and whose disconnection has been found to be implicated in schizophrenia: the UF and the SLF III. These are relatively long associative fibers, among the major connections to the frontal cortex (Crosby et al., 1962), whose growth and extended maturation in the brain during fetal life and childhood make them plausible targets for the substrate of a neurodevelopmental disorder (Jones et al., 2006). Particularly, the UF is a ventral limbic pathway that links the rostral superior temporal gyrus, the rostral inferior temporal gyrus, the medial temporal area (entorhinal, perirhinal, parahippocampal gyri), and the orbital, medial and prefrontal cortices. We found that significant predictors of lower FA in UF were CROP score and number of MWCST categories. Thus, UF's connections are likely to underlie the cognitive processes involved in both the above-mentioned neuropsychological tasks.

As well as the UF, the SLF III is implicated in schizophrenia (Burns et al., 2003). We found that significant predictors of FA in SLF III were CROP and n-back verbal scores. These results are consistent with the anatomy of this fiber bundle, which links the rostral inferior parietal lobule, the supramarginal

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**Table 4**

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Fiber</th>
<th>White matter</th>
<th>Gray matter</th>
<th>Coordinates</th>
<th>Cluster size</th>
<th>p value*</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
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<td>gyrus</td>
<td>MNI</td>
<td>x  y  z</td>
<td></td>
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<tr>
<td>Cluster 7</td>
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<td>SLF III</td>
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<td>36</td>
<td>14</td>
</tr>
<tr>
<td></td>
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<td>Triangularis</td>
<td>(BA 45)</td>
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<td>24</td>
<td>0</td>
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<tr>
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<td>pars</td>
<td>(BA 45)</td>
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<td>16</td>
</tr>
<tr>
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<td>frontal</td>
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<td>34</td>
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<tr>
<td>Cluster 6</td>
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<td>16</td>
</tr>
<tr>
<td></td>
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<td>pars</td>
<td>cingulum</td>
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<td>-80</td>
<td>18</td>
</tr>
</tbody>
</table>

*p value corrected Family Wise Error.

SLF III: Superior Longitudinal Fasciculus subcomponent III; UF: Uncinate Fasciculus.
gyrus (an intramodal association area concerned with high order somatosensory information), the pars opercularis, and the ventral prefrontal area, which is engaged in working memory. Furthermore, the finding that verbal working memory is a significant predictor of SLF III reduced WM integrity is consistent with a recent DTI study (Karlsgodt et al., 2008) as well as with evidence of frontal parietal activation deficit during working memory tasks in patients with schizophrenia (Barch and Csernansky, 2007).

In their entirety, these results suggest that abnormalities in WM microstructure integrity in the above-mentioned fiber tracts are linked to frontal cognitive deficits in schizophrenia. Thus, we may speculate that the anisotropy alterations related to the functional changes in brain connectivity may be involved in the neuropathology of cognitive impairment in schizophrenia.

4.1.1. Right hemisphere hypo-connectivity in schizophrenia

All the reduced FA clusters, with the exception of the EmC (i.e. ILF, SLF III, and UF), are right-lateralized, seemingly in contrast with part of the existing literature (Burns et al., 2003; Kubicki et al., 2002). Nevertheless, as discussed by Mitchell and Crow (2005), the importance of the right hemisphere in schizophrenia has been disregarded for a long time, and previous authors have argued that the right hemisphere plays a role in the pathophysiology of schizophrenia (Cutting, 1994; David, 1994). In fact, many nuclear symptoms of schizophrenia have been found to be associated with right hemisphere dysfunction, e.g. auditory hallucinations (Jaynes, 1979; Nasrallah, 1985) or thought insertion and social withdrawal, which are linked to activation and deactivation in right dorsolateral prefrontal cortex (Crow, 1998a, 2004a). Disorganization of speech has been understood to be a product of the interactions between the dorsolateral prefrontal cortex and occipito-temporo-parietal cortex in the right hemisphere (Crow, 1997, 2004b). Also emotional communication skills (i.e. prosody) and the interpretation of others' emotional state in order to direct social behaviour (affect recognition, a social cognitive ability which is impaired in schizophrenia) (Pinkham et al., 2007) are lateralized in the right hemisphere (Mitchell and Crow, 2005). Finally, from a neuropsychological point of view, alertness, a fundamental component of attention (Callejas et al., 2005; Posner and Petersen, 1990) is thought to be subserved by a mostly right-lateralized frontal, parietal, thalamic and brainstem network (Sturm and Willmes, 2001).

Fig. 2. Correlation between white matter fractional anisotropy and gray matter density in 43 schizophrenic patients. Correlations between white matter microstructure and gray matter density are shown in Panel A and B. Panel A: a decrease of FA in right UF (in blue) was significantly associated with a decrease of gray matter density in the right Anterior Cingulum and the right Cerebellum (in red). Panel B: a decrease of FA in right SLF III (in blue) was significantly associated with a decrease of gray matter density in the Right MFG, the right Pars Triangularis, and the Left MFG (in red). All the results were overlaid on the template for a better visualisation of the regions involved. L: Left; R: Right; SLF III: Superior Longitudinal Fasciculus subcomponent III; UF: Uncinate Fasciculus; MFG: Middle Frontal Gyrus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Thus, our result of a mainly right-lateralized WM integrity reduction and its above-mentioned cognitive correlates provides additional evidence of a key role of the right hemisphere in schizophrenia.

4.1.2. Increased FA in Internal Capsule, posterior limb

Our finding of an increased FA in the ICp bilaterally is a novel result. Despite the neuropathological counterpart of an increased FA is not fully understood, it could probably be underpinned by abnormal axonal migration causing alterations in axonal packing density. From research on autism we know that an increased WM volume in primary motor and premotor areas is linked to motor impairment and to deficits in socialization and communication (Mostofsky et al., 2007), thus we may hypothesize that an increased anisotropy in the ICp, because of its connections to those areas, could be linked to abnormal motor and/or communicative behaviours in schizophrenia. However, exploring this issue was not among the aims of this study, and further research is needed to validate this hypothesis.

4.2. Anatomically related gray and white matter abnormalities

The VBM-style analysis indicates that WM in 2 out of the 6 clusters of reduced FA is positively correlated with reduced GM density in neuroanatomically-related regions. Particularly, FA values in the right UF correlated with GM density in the right anterior cingulum and the right cerebellum; and FA values in the SLF III correlated with GM density in the right pars triangularis and the middle frontal gyrus bilaterally. We explain these findings considering the anatomical connections of these structures and their functional meaning.

The UF comprises the cingular bundle, which is the most prominent fiber tract within the limbic system, and its anterior–agranular, motor-related cortex is interconnected to amygdala, nucleus accumbens, medial dorsal thalamus, and dorsolateral prefrontal cortex. The anterior cingulate cortex has been found to be implicated in schizophrenia (Carter et al., 1998; Kiehl et al., 2000), and linked to cognitive deficits (i.e. error detection processing, decision monitoring, and attention) (Cohen et al., 1998; Nestor et al., 2007), positive symptoms (i.e. hallucinations) (Frith, 1995), and social cognitive processing (i.e. affect recognition) (Tammenga et al., 2000). Previous studies have described a reduced GM volume in the anterior cingulate gyrus in schizophrenia (Farrow et al., 2005; Jayakumar et al., 2005; Job et al., 2002; Kubicki et al., 2002; Salgado-Pineda et al., 2003). Furthermore, a disruption in the limbic system network integrity, and in particular in the cingulate gyrus, has been hypothesized to be involved in the pathophysiology of schizophrenia, explaining some of the psychiatric and cognitive symptoms (Cohen et al., 1999). The cerebellum has been found to show a reduced GM in schizophrenia (Farrow et al., 2005; Jayakumar et al., 2005; Salgado-Pineda et al., 2003) and to play an important role in cognitive processes, neurological soft signs, dyscoordination, abnormal posture and proprioception, impaired eyeblink conditioning (Picard et al., 2008). Furthermore, enlarged cerebellar ventricles, including lateral and third ventricles, are associated with deficits in attention, executive, and premorbid cognitive functioning in schizophrenic patients (Crespo-Facorro and Barbadillo, 2007), consistently with the above-described neuropsychological predictors of reduced FA in UF in this study. Thus, it could be hypothesized that the reduced WM integrity in the UF is underpinned by structural changes in these two cingular and cerebellar regions. More research is needed in order to substantiate this hypothesis.

Similarly, the topography of GM atrophy in the middle frontal gyri bilaterally and the right pars triangularis suggests a structural substrate for the impairment in WM integrity in the right SLF III. The pars triangularis is a triangular-shaped aspect of the gyral structure in the lateral and inferior part of frontal lobe situated between pars opercularis and pars orbitalis. We previously found a reduction in GM volume in left pars triangularis in schizophrenia (Spalletta et al., 2003). The middle frontal gyrus, which is located on the lateral surface of the temporal lobe ventral to the superior temporal gyrus, has been found to show a reduced GM in schizophrenia (Jayakumar et al., 2005; Onitsuka et al., 2004), and to be involved in several cognitive processes (Cabeza and Nyberg, 2000). Thus, we speculate that the reduced right SLF III WM integrity may be underpinned by structural changes in the right pars triangularis and bilateral middle frontal gyri, representing the basis for an impairment in neuropsychological abilities, such as working memory and complex constructive-praxis tasks, as previously argued.

4.3. Strengths and limitations of the study and conclusions

The main strength of this study is the use of a refined VBM-like approach for GM analysis and the method of TBSS for WM analysis, performed on the same sample of schizophrenic patients.

However, some limitations need to be taken into account. Firstly, results of differences in anisotropy within WM fibers are open to a number of interpretations, as the data would be consistent with either the presence of differences in number and/or architecture of the axons (which are expressed by one out of the two FA constituent parameters, i.e. Axial Diffusivity, indicating parallel diffusion), or in myelination of tracts and/or alteration in the axonal cytoskeleton (expressed by the FA perpendicular constituent parameter, i.e. Radial Diffusivity), or a combination of such processes (Beaulieu, 2002). From a very recent DTI study in schizophrenic patients (Seal et al., 2008) using TBSS, we know that FA reduction in SLF, UF and other WM tracts, is accounted for by an increase in Radial Diffusivity but not in Axial Diffusivity, thus it is likely to be underpinned by reduced axonal myelination rather than gross axonal damage. Further studies should aim to clarify the nature of the WM microstructure abnormalities in schizophrenia.

Secondly, it is not possible to specify if WM reduction is caused by GM reduction or vice versa, as neither the directionality nor the pathophysiology of the associations between lower WM integrity and lower GM density is fully understood. More research is needed in order to clarify this issue.

Thirdly, our sample is represented by non-first-onset schizophrenic patients, and there is evidence of a positive association between reduced WM integrity and illness duration in schizophrenia patients (Walterfang et al., 2006). However, illness duration was included in all the stepwise multiple regression analyses and was not a significant predictor of any FA clusters in this study.

Fourthly, all schizophrenic patients in our study received cumulative doses of atypical antipsychotics, and an influence of chronic treatment on the results should be taken into
consideration (Bartzokis et al., 2007; McCormick et al., 2005; Scherk and Falkai, 2006). However, we performed a series of univariate correlation analyses which did not show any significant association between olanzapine equivalents and FA value for each cluster (data available upon request). Therefore, we exclude a significant confounding effect of medication on our sample results.

Finally, as schizophrenic patients and HC differed for educational attainment, one may wonder if education could represent a confounding variable on the results. However, performing a series of univariate correlation analyses we failed to find any significant association between education and FA value for each cluster (data available upon request). Thus, we also exclude a confounding effect of educational attainment on our sample results. Further research is necessary to deepen all these issues.

In conclusion, we demonstrate a significant reduction in WM integrity in fronto-temporal fiber tracts and their neuropsychological predictors in schizophrenic patients. The topographic distribution of the WM differences is similar to those reported previously in literature but more right-lateralized, consistently with Mitchell and Crow’s hypothesis (Mitchell and Crow, 2005). Furthermore, some of these WM abnormalities are associated with reduced GM density in neuroanatomically related regions. Considered comprehensively, our data further support the disconnectivity hypothesis in schizophrenia (McClaslan and Hoffman, 2000; Stephan et al., 2006), suggesting a link between reduced fronto-temporal connectivity and cognitive deficits in typically impaired domains in schizophrenia, as well as structural GM reduction.

These results help to clarify structural and connectivity disturbances and their relation with frontal cognitive symptoms in schizophrenia, leading to a more comprehensive neuroanatomical characterization of this complex disorder.

Role of the funding source
This research has been supported by the RC 05/A grant from the Italian Ministry of Health.

Contributors
Gianfranco Spalletta, MD, PhD, wrote the protocol, designed the study, took part at the data collection process and undertook the statistical analyses. Ilaria Spiletini, PhD, wrote the first draft of the manuscript and took part at the data collection process. Andrea Cherubini, PhD, and Margherita di Paola, BS, managed the literature searches and analyses. Giulia Banfi, BS, Nicola Rüsch, MD, Giovanni Martinotti, MD, Ivo Alex Rubino, MD, and Alberto Ilaria Spoletini, PhD, wrote the first draft of the manuscript and took part at the data collection process. Carlo Caltagirone, MD, and Pietro Bria, MD, designed the study. All authors have contributed to and have approved the final manuscript.

Dr. Gianfranco Spalletta has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest
The authors have no conflict of interest.

Acknowledgements
No acknowledgements.

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