Hippocampi, Thalami, and Accumbens Microstructural Damage in Schizophrenia: A Volumetry, Diffusivity, and Neuropsychological Study

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Volumetric abnormalities in the subcortical structures have been described in schizophrenia. However, it still has to be clarified if subtle microstructural damage is also present. Thus, we aimed to detect subcortical volume and mean diffusivity (MD) alterations in 45 patients with diagnosis of schizophrenia compared with 45 age-, gender-, and educational attainment–matched healthy comparison (HC) participants, by using a combined volumetry and diffusion tensor imaging (DTI) method. A secondary aim was to identify the neuropsychological correlates of subcortical abnormalities in the schizophrenic group. We found thalami and hippocampi bilaterally and left accumbens to show MD increase in the schizophrenic group. No volumetric decrease was found. Moreover, significant correlations between the MD values in subcortical structures (right thalamus and hippocampus and left accumbens) and working memory performance were found. Thus, subcortical microstructural alterations are present in schizophrenia even in absence of volumetric abnormalities. Furthermore, microstructural damage in subcortical areas is linked to working memory, suggesting the presence of a subtle microstructural subcortical dysfunction in the pathoetiologic mechanism underlying high cognitive load performances in schizophrenia. Finally, our findings indicate that MD is a more sensitive marker of brain tissue deficits than signal intensity variations measured in T1-weighted imaging data, consistently with previous reports. Thus, DTI appears to be an invaluable tool to investigate subcortical pathology in schizophrenia, greatly enhancing the ability to detect subtle brain changes in this complex disorder.

Keywords: subcortical structure/mean diffusivity/volume/working memory

Introduction

Neuroimaging studies suggest that volumetric abnormalities characterize subcortical areas, especially thalamus1–4 and hippocampus,5,6 in schizophrenia patients. However, it still has to be clarified if these structural alterations are associated with subtle damage in the tissue microstructure. Furthermore, because schizophrenia is a mental disorder often associated with cognitive impairment,7 it is important to clarify if subcortical volumetric and microstructural abnormalities are also associated with neuropsychological performances.

Here, we opted to use different neuroimaging techniques to provide complementary information of microstructural and volumetric changes within the subcortical areas. Particularly, because diffusion tensor imaging (DTI) is sensitive to microscopic random motion of water molecules, it is able to detect subtle microstructural abnormalities in different cerebral tissues8,9 providing functional or physiologic information not present on conventional T1- and T2-weighted anatomic magnetic resonance (MR) images.10 An index that can be obtained by DTI is mean diffusivity (MD), a quantitative measure of directionally averaged diffusion. An increase in MD is thought to reflect an enlargement in the extracellular space due to altered cytoarchitecture, suggesting immaturity or degeneration of the tissue.11 Although DTI has been used mainly to investigate regional white matter changes, it can be also utilized to highlight microstructural alterations of subcortical gray matter,12 as demonstrated by previous reports on schizophrenia.13,14 Hence, by combining the study of atrophy/hypertrophy in MR volumetry with the potential of DTI in highlighting microstructure alterations, it should be possible to describe in detail subcortical abnormalities in schizophrenia. Furthermore, the high-resolution MR images acquired for volumetric methods should facilitate the identification of anatomical structures with limited spatial extension, such as the subcortical nuclei and gray matter structures.
Bearing these considerations in mind, the first aim of this study was to probe the volume and tissue microstructure of subcortical areas in patients with diagnosis of schizophrenia without global cognitive impairment in comparison with age-, gender-, and educational attainment–matched healthy comparison (HC) subjects. Here, the term “subcortical” is used in a broad sense to refer to the subcortical nuclei as well as to gray matter limbic regions such as amygdala and hippocampus. To our knowledge, no neuroimaging studies in schizophrenia so far have investigated subcortical microstructure and volume at the same time in these areas. In addition, in this kind of study, it is important to consider patients without global cognitive impairment because it has been described as a potential confounding variable on the subcortical volume, specifically of the hippocampus, and on the whole-brain volume.7,16

An optimized methodology by combining magnetic resonance imaging (MRI) volumetry and DTI was applied to the subcortical areas. In order to precisely identify the structures of interest, we employed a fully automated, model-based segmentation algorithm on MRI anatomical images and accurate coregistration of DTI to anatomical images to extract regional values of DTI parameters in these specific areas.

Following the hypothesis that the microarchitectural damage may affect the tissue in a given subcortical structure before the volumetric alterations are detectable, as preliminarily and selectively demonstrated by Kalus et al 17 on the hippocampus and by Agarwal et al 18 on the thalamus, we expected widespread subtle alterations in subcortical microstructural integrity (indicated by increase in MD) to characterize our sample of patients with diagnosis of schizophrenia without global cognitive impairment even in absence of volumetric abnormalities.

A secondary aim of this study was to investigate the neuropsychological correlates of MD and volume alterations in subcortical areas in the schizophrenic group. Considering that, among the other cognitive abilities, working memory and executive function are greatly impaired in schizophrenia,19,20 we hypothesized microstructural and/or volume abnormalities in the subcortical areas to be related to working memory and executive function domains.

Methods

Subjects

Seventy-five consecutive patients with a diagnosis of schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) (DSM-IV) 21 were initially recruited from 2 outpatient clinics in central Italy. Diagnosis of schizophrenia was independently performed by 2 clinicians, who had been trained until an interrater reliability level of $k \geq 0.80$ was reached. Specifically, a clinician (I.A.R.), who treated the patients and knew their clinical history, used DSM-IV criteria to make a preliminary diagnosis of schizophrenia. The clinician was blind to the aims of the study. All diagnoses were then confirmed, or not, the day of image acquisition, by another clinical psychiatrist (G.S.) using the Structured Clinical Interview Patient Edition for DSM-IV (SCID-P).22 The second psychiatrist also screened the patients according to the inclusion and exclusion criteria of the study. In the case of disagreement between the 2 psychiatrists, more data were requested to help resolve the differences, and the diagnostic process continued until a final consensus diagnosis was assigned. If there was still disagreement among the 2 diagnosticians, the patient was removed from the sample.

Severely of schizophrenia symptoms was assessed by using the Positive and Negative Syndrome Scale (PANSS)23 (mean total PANSS score $\pm$ SD = 91.96 ± 21.87, mean positive symptoms score $\pm$ SD = 22.11 ± 7.19, mean negative symptoms score $\pm$ SD = 21.22 ± 7.73, mean general psychopathology score $\pm$ SD = 48.62 ± 12.41). 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 participants included were between 18 and 66 years of age, were right-handed, and had completed at least 5 years of schooling. An inclusion criterion was the Mini-Mental State Examination (MMSE)24 score equal to or higher than 25 in order to avoid the inclusion of patients with global cognitive deterioration.

Exclusion criteria were history of alcohol or drug dependence or traumatic head injury, any past or present major medical or neurological illness (in particular, dementia diagnosis was accurately excluded by using a comprehensive neuropsychological battery, which is described in the cognitive examination section), any additional psychiatric disorder, any brain pathology identified on T2 or fluid attenuated inversion recovery scans, and mental retardation. All patients were receiving stable oral doses of one or more atypical antipsychotic drugs such as risperidone, quetiapine, and olanzapine. Antipsychotic dosages were converted to estimated equivalent dosages of olanzapine.25

From the 75 patients of the initial sample, 30 patients were excluded according to the above-mentioned exclusion criteria. Specifically, 15 patients showed global cognitive impairment (ie, a MMSE score lower than 25), 8 patients had history of alcohol and/or psychoactive drug dependence, 2 had traumatic head injury, 1 suffered from mental retardation, 2 met criteria for other psychotic disorders according to the diagnosis made by the psychiatrist who administered the SCID-P (1 delusional disorder, 1 schizoaffective disorder), and 2 patients were removed from subsequent analyses due to significant movement artifacts during the MRI procedure. Thus, the final sample consisted of 45 patients with diagnosis of schizophrenia.
Forty-five HC subjects were recruited in the same geographic area and rigorously matched with the patients with diagnosis of schizophrenia for age, gender, and educational attainment (table 1). All the HC subjects were carefully screened for a current or past diagnosis of any Axis I or II disorder using SCID-I or SCID-II, respectively. Schizophrenia or any other mental disorder diagnosis among first-degree relatives was an exclusion criterion as well as the other above-mentioned exclusion criteria for patients.

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

**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 7 days of MRI. All the patients and HC participants underwent the cognitive examination. Forty-two out of the 45 HC subjects completed the entire neuropsychological assessment (data available upon request). Acceptable interrater reliability level for the present study was defined as of $k > 0.80$.

As previously mentioned, we selected a group of patients with diagnosis of schizophrenia without global cognitive impairment by administering the MMSE, as it is one of the most common instruments used to screen for cognitive deterioration. It is also brief and easy to administer and has been widely applied in research on schizophrenia. MMSE provides a global index of cognitive impairment by measuring orientation, language, verbal memory, attention, visuospatial function, and mental control. It is composed of 16 items, with scores ranging from 30 (no impairment) to 0 (maximum impairment). Specific neuropsychological domains were also assessed by administering tests from the Mental Deterioration Battery (MDB). The MDB is a standardized and validated neuropsychological battery, comprising neuropsychological tests pertaining to the elaboration of verbal and visuospatial materials. Three tests were selected from MDB in order to provide information about the functionality of verbal memory (Rey’s 15-Word Immediate Recall [RIR] and Delayed Recall [RDR]) and logical reasoning (Raven’s Progressive Matrices’ 47 [PM47]). Furthermore, we used the copy of Rey-Osterrith complex picture (ROPC) and its delayed recall, which provide information—respectively—about visual perception/constructural praxis and long-term visuospatial memory.

Following the aims of the study, and considering that “executive functioning” denotes a set of different cognitive abilities involved in complex, goal-directed thought and behavior, the following executive dimensions were assessed: (a) attentional control/inhibition, (b) set shifting, and (c) working memory.

In order to measure abilities of attentional control and inhibition, we administered the Stroop Test (ST). The executive dimension of set shifting or cognitive flexibility, ie, the ability to alter a behavioral response mode in the face of changing contingencies, was assessed using the Modified Wisconsin Card Sorting Test (MWCST). Participants were also administered the Trail Making Test (TMT) to assess cognitive flexibility and ability to shift strategy.

Finally, in order to measure verbal, spatial, and visual working memory, we administered the “n-back” tasks. The n-back test has been widely used in previous research. In this test, subjects are required to monitor

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients With Diagnosis of Schizophrenia (N = 45)</th>
<th>Comparison Subjects (N = 45)</th>
<th>t</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39.8 ± 11.02</td>
<td>39.47 ± 17.10</td>
<td><strong>−0.110</strong></td>
<td><strong>88</strong></td>
<td><strong>.9127</strong></td>
</tr>
<tr>
<td>Educational level (y)</td>
<td>13.04 ± 2.36</td>
<td>13.47 ± 3.17</td>
<td><strong>0.716</strong></td>
<td><strong>88</strong></td>
<td><strong>.4761</strong></td>
</tr>
<tr>
<td>Age at the onset of the illness (y)</td>
<td>26.24 ± 8.37</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Duration of the illness (y)</td>
<td>13.56 ± 10.50</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Olanzapine equivalents (mg/d)</td>
<td>20.16 ± 32.93</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>25 (55)</td>
<td>25 (55)</td>
<td><strong>χ²</strong></td>
<td><strong>1</strong></td>
<td><strong>&gt;.9999</strong></td>
</tr>
<tr>
<td><em>DSM-IV</em> subtypes</td>
<td></td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Paranoid schizophrenia</td>
<td>30 (67)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Disorganized schizophrenia</td>
<td>2 (4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Undifferentiated schizophrenia</td>
<td>5 (11)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Residual schizophrenia</td>
<td>8 (18)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Note: DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition).*
continuously a sequence of verbal/spatial/visual stimuli (a total of 22 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 that is differentially located among black boxes; and finally, in the visual task, stimuli are a series of abstract pictures.

The item selection was done by the participants using a keyboard with 3 keys, 1 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 (ie, verbal/spatial/visual) at 3 different levels of difficulty. At the “n-1 level,” subjects were required to select an item that appears 1 item back in a sequence, at the “n-2 level” to select an item that appears 2 items back in a sequence, and at the “n-3 level” to select an item that appears 3 items back in a sequence. The number of correct responses (“accuracy score”) was 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 sequence 3 times before starting the experimental procedure. Accuracy scores as memory load at the n-2 level for the 3 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.

Image Acquisition

The 90 participants underwent the same imaging protocol with a whole-brain T1-weighted and diffusion-weighted scanning using a 3-T Allegra 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 sequence (echo time [TE]/repetition time [TR] = 2.4/7.92 ms, flip angle = 15°, voxel size = 1 × 1 × 1 mm³).

Diffusion-weighted images were obtained using echo planar imaging (spin-echo planar imaging, TE/TR = 89/8500 ms, 52 axial slices, bandwidth = 1860 Hz per voxel, 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/mm² and 2 b = 0 images.

All image processing was performed using FSL 4.0 software. 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 no diffusion-weighted image. Nevertheless, it should be noted that the mean b₀ image used for eddy current corrections suffered from distortions itself; thus, all the typical artifacts present in echo planar images cannot be completely removed using this procedure. After distortion corrections, DTI data were averaged and concatenated into 13 (1 b₀ + 12 b₁₀₀₀) volumes. A diffusion single-tensor model was fit at each voxel, generating MD and fractional anisotropy (FA) maps. The FA images were used to obtain a better coregistration with T1-weighted images because the spatial distribution of signal intensities was similar in both image modalities, while MD values were used as an index of structural integrity within subcortical nuclei. The FA maps created were then registered to brain-extracted whole-brain volumes from T1-weighted images using a full affine (correlation ratio cost function) alignment with nearest-neighbor resampling. The calculated transformation matrix was then applied to the MD maps with identical resampling options. For each subject, the coregistered FA map was superimposed to the original T1-weighted volume, and the resulting images were visually assessed by a trained radiologist.

Image Processing. Anatomical T1-weighted images were processed with the segmentation tool FIRST 1.0 integrated within the FSL software. FIRST (http://www.fmrib.ox.ac.uk/fsl/first/index.html) is a completely automatic, model-based segmentation/registration tool. The shape/appearance models used in FIRST are constructed from manually segmented images provided by the Center for Morphometric Analysis, Massachusetts General Hospital, Boston, MA. The manual labels are parameterized as surface meshes and modeled as a point distribution model. Deformable surfaces are used to automatically parameterize the volumetric labels in terms of meshes; the deformable surfaces are constrained to preserve vertex correspondence across the training data. Furthermore, normalized intensities along the surface normals are sampled and modeled. The shape-and-appearance model is based on multivariate Gaussian assumptions. Shape is then expressed as a mean with modes of variation (principal components). Based on learned models, FIRST searches through linear combinations of shape modes of variation for the most probable shape instance given the observed intensities in the T1 image. FIRST uses mesh models trained with a large amount of rich hand-segmented training data to segment subcortical structures. This method of segmentation is particularly useful for structures with low contrast-to-noise ratio. In each subject, the lateral ventricles, caudate, thalamus, hippocampus, putamen, accumbens, pallidum, and amygdala were segmented. For each subject, the segmentation results were visually assessed by a trained radiologist.

For each subject and each hemisphere, we calculated the volumes of the 8 above-mentioned segmented subcortical areas. Before statistical comparison, individual volume values were multiplied by a normalization factor.
obtained with the SIENAX tool\textsuperscript{48} from the corresponding T1-weighted image. A 2-sample $t$ test was performed in order to verify that the 2 groups did not differ for the normalization factor ($P = .8867, t = -0.1429, SD = 0.0356$, Cohen $d = 0.0299$).

These segmented structures defined the region of interest from where mean and SD of MD values were calculated for each individual and for each hemisphere.

\textit{Statistical Analyses}

Data from all 90 subjects were included in statistical analyses. Comparisons between the 2 groups on age and educational level were performed using $t$ tests. Comparison between the 2 groups on gender variable was performed using the $\chi^2$ test.

In order to minimize the likelihood of type I error, a series of univariate analyses of variance (ANOVAs) was preceded by overall multivariate analysis of variance (MANOVA) using all the continuous categories considered in each of the analyses as dependent variables. Consequently, 1-way ANOVAs (between groups) were run for each volume and diffusivity parameter of each area. A level of significance of $P < .05$ for comparative measurements was used throughout the study.

\textit{Correlation Between Subcortical Volume, MD, and Neuropsychological Scores in the Schizophrenic Group.} Given the large number of neuropsychological variables here assessed (ie, 12), the principal component analysis (PCA) was used as the extraction procedure to identify underlying neuropsychological factors, after conversion of raw test scores into standard equivalents (ie, $z$ scores) using test means and SDs. Bartlett index of sphericity was checked to assess the appropriateness of factor analysis. Eigenvalues $\geq 1$ were used as guides to establish the number of factors. Factor loadings greater than 0.50 were added. An orthogonal rotational procedure (varimax) enabled the improvement of the interpretation of factors. In order to make the underlying factor structure clearer, by extracting as high differences between the variables as possible, an additional transformation, the orthotran transformation, was applied.\textsuperscript{49,50} Two matrices were generated: the “primary pattern matrix” and the “reference structure matrix.” The reference structure matrix was chosen because it shows by definition correlations among variables in a clearer way.\textsuperscript{49} Factorial scores were saved for successive analysis. Univariate correlation analyses (Pearson $r$) and Fisher’s $r$-to-$z$ transformation were then computed between the cognitive factors yielded by the PCA and the diffusivity and volume scores previously found to differentiate patients and controls.

\textit{Results}

As expected from the matching procedure, patients with diagnosis of schizophrenia and HC subjects did not significantly differ for age, gender, and educational attainment (table 1).

\textit{Volumetric and DTI Data}

An automatic segmentation method\textsuperscript{47} was used for delineating the lateral ventricles and 7 subcortical structures (thalamus, hippocampus, caudate, putamen, pallidum, amygdala, and accumbens) on each subject (see figure 1).

In table 2, MD values of the subcortical structures are reported. A MANOVA using all the MD scores as dependent variables (Wilks $\lambda = 0.712; F = 2.172; df = 14,75; P = .0167$) and a series of follow-up 1-way ANOVAs revealed a significant increase of MD in the right and left thalami, right and left hippocampi, and left accumbens in patients with diagnosis of schizophrenia compared with HC subjects, as shown in figure 2.

In table 3, subcortical volumes are indicated. A MANOVA using all the subcortical volume scores as dependent variables indicated that volumes of subcortical areas were globally different between patients and HC subjects (Wilks $\lambda = 0.692; F = 2.030; df = 16,73; P = .0167$) and a series of follow-up 1-way ANOVAs revealed a significant increase of MD in the right and left thalami, right and left hippocampi, and left accumbens in patients with diagnosis of schizophrenia compared with HC subjects. On the contrary, we found increased volumes in the left
accumbens. Interestingly, left ventricle was enlarged in patients with diagnosis of schizophrenia. Furthermore, 2 additional MANOVAs for both MD and volume values were performed. In a first MANOVA, values from left and right brain subcortical structure averaged (ie, a sum of each bilateral structure divided by 2) were used in order to explore whether the means differed across groups. In a second MANOVA, we used the “differenced values” (ie, obtained by subtracting left brain subcortical values from right values) in order to further explore whether hemispheric asymmetry differed across groups. Consistently with our previous results, averaged left and right brain subcortical values were significantly different between patients and HC groups for both MD and volume values, respectively (Wilks $\lambda$ = 0.772; $F$ = 3.462; $df$ = 7,82; $P$ = .0027; and Wilks $\lambda$ = 0.810; $F$ = 2.370; $df$ = 8,81; $P$ = .0240). A series of follow-up 1-way ANOVAs revealed a significant increase of MD in the averaged thalami, hippocampi, and accumbens of patients, whereas no individual volumetric differences were found in patients with diagnosis of schizophrenia compared with HC subjects (data available upon request). On the other hand, the results from the other two MANOVAs showed no significant differences between patients and HC groups for the differenced MD and volume values ($P$ > .175 for both comparisons).

**Cognitive Correlates of Subcortical Abnormalities in Schizophrenia**

Neuropsychological scores were computed by using a PCA, incorporating the 12 above-described neuropsychological scores. PCA extracted 4 factors with eigenvalues greater than 1, representing a total of 77.4% of the

<table>
<thead>
<tr>
<th>Structures</th>
<th>Side</th>
<th>Patients With Diagnosis of Schizophrenia Mean (SD)</th>
<th>HC Subjects Mean (SD)</th>
<th>ANOVA $F$ ($df$: 1,88)</th>
<th>$P$</th>
<th>Cohen $d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td>Right</td>
<td>870 (33)</td>
<td>851 (42)</td>
<td>5.63</td>
<td>.0198</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>845 (29)</td>
<td>824 (35)</td>
<td>10.07</td>
<td>.0021</td>
<td>0.67</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Right</td>
<td>984 (54)</td>
<td>948 (35)</td>
<td>14.36</td>
<td>.0003</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>993 (53)</td>
<td>954 (34)</td>
<td>17.05</td>
<td>.0001</td>
<td>0.87</td>
</tr>
<tr>
<td>Caudate</td>
<td>Right</td>
<td>998 (104)</td>
<td>964 (101)</td>
<td>2.56</td>
<td>.1129</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>930 (79)</td>
<td>904 (76)</td>
<td>2.58</td>
<td>.1117</td>
<td>0.34</td>
</tr>
<tr>
<td>Putamen</td>
<td>Right</td>
<td>769 (41)</td>
<td>761 (43)</td>
<td>0.83</td>
<td>.3636</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>759 (37)</td>
<td>749 (29)</td>
<td>1.89</td>
<td>.1731</td>
<td>0.29</td>
</tr>
<tr>
<td>Pallidum</td>
<td>Right</td>
<td>728 (68)</td>
<td>742 (44)</td>
<td>1.35</td>
<td>.2488</td>
<td>−0.24</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>730 (56)</td>
<td>744 (48)</td>
<td>1.69</td>
<td>.1975</td>
<td>−0.27</td>
</tr>
<tr>
<td>Amygdala</td>
<td>Right</td>
<td>971 (80)</td>
<td>954 (68)</td>
<td>1.18</td>
<td>.2796</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>945 (67)</td>
<td>929 (60)</td>
<td>1.32</td>
<td>.2536</td>
<td>0.24</td>
</tr>
<tr>
<td>Accumbens</td>
<td>Right</td>
<td>771 (62)</td>
<td>754 (52)</td>
<td>1.97</td>
<td>.1641</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>772 (60)</td>
<td>742 (46)</td>
<td>7.02</td>
<td>.0095</td>
<td>0.56</td>
</tr>
</tbody>
</table>

*Note: Significant $P$ values ($P < 0.05$) are indicated in bold. HC, healthy comparison; ANOVA, analysis of variance.*

Fig. 2. Average Reconstruction of the Subcortical Regions That Showed Significant Group Differences Between Patients and Healthy Controls. The bar graphs report the average mean diffusivity values ($\times 10^{-6}$ mm$^2$/s) and SEs for individual left or right structures.
variance. The orthotran transformation generated 2 matrices. As expected, factors in the reference structure matrix were equivalent to those generated using the primary pattern matrix, in the sense that the same variables fitted in the same factors. This is intuitive because one is a column rescaling of the other.\(^{49}\) We considered as results the factors shown by the reference structure matrix, which is reported in Table 4.

### Table 3. Normalized Volumes (mm\(^3\)) of the Subcortical Areas in 45 Patients With Diagnosis of Schizophrenia and 45 HC Subjects

<table>
<thead>
<tr>
<th>Area</th>
<th>Side</th>
<th>Mean (SD) Patients With Diagnosis of Schizophrenia</th>
<th>Mean (SD) HC Subjects</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F (df: 1, 88) P Cohen d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral ventricles</td>
<td>Right</td>
<td>6445 (2892)</td>
<td>5505 (3421)</td>
<td>1.98 0.1629 0.30</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>6795 (3395)</td>
<td>5445 (2916)</td>
<td>4.09 0.0460 0.43</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Right</td>
<td>6107 (730)</td>
<td>6308 (666)</td>
<td>1.85 0.1777 –0.29</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>5697 (706)</td>
<td>5979 (706)</td>
<td>3.60 0.0612 –0.40</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Right</td>
<td>2560 (365)</td>
<td>2630 (297)</td>
<td>0.98 0.3237 –0.21</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>2401 (285)</td>
<td>2452 (318)</td>
<td>0.64 0.4255 –0.17</td>
</tr>
<tr>
<td>Caudate</td>
<td>Right</td>
<td>2738 (422)</td>
<td>2610 (471)</td>
<td>1.85 0.1777 0.29</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>2670 (460)</td>
<td>2556 (460)</td>
<td>1.37 0.2441 0.25</td>
</tr>
<tr>
<td>Putamen</td>
<td>Right</td>
<td>3782 (472)</td>
<td>3725 (428)</td>
<td>0.37 0.5461 0.13</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>3619 (521)</td>
<td>3634 (489)</td>
<td>0.02 0.8917 –0.03</td>
</tr>
<tr>
<td>Pallidum</td>
<td>Right</td>
<td>1026 (228)</td>
<td>1092 (180)</td>
<td>2.29 0.1338 –0.32</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>1044 (184)</td>
<td>1056 (150)</td>
<td>0.11 0.7388 –0.07</td>
</tr>
<tr>
<td>Amygdala</td>
<td>Right</td>
<td>818 (155)</td>
<td>813 (190)</td>
<td>0.02 0.9011 –0.03</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>750 (147)</td>
<td>806 (169)</td>
<td>2.81 0.0969 –0.35</td>
</tr>
<tr>
<td>Accumbens</td>
<td>Right</td>
<td>235 (73)</td>
<td>215 (81)</td>
<td>1.53 0.2189 0.26</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>320 (98)</td>
<td>281 (87)</td>
<td>3.98 0.0491 0.42</td>
</tr>
</tbody>
</table>

Note: Significant \(P\) values (\(P < 0.05\)) are indicated in bold. HC, healthy comparison, ANOVA, analysis of variance.

### Table 4. Principal Component Analysis of the Neuropsychological Variables in 45 Patients With Diagnosis of Schizophrenia: Orthotran Reference Structure Matrix

<table>
<thead>
<tr>
<th>Variables</th>
<th>Independent Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factor 1 (Working Memory)</td>
</tr>
<tr>
<td>RIR</td>
<td>0.036</td>
</tr>
<tr>
<td>RDR</td>
<td>-0.041</td>
</tr>
<tr>
<td>PM47</td>
<td>-0.062</td>
</tr>
<tr>
<td>TMT ratio score</td>
<td>0.059</td>
</tr>
<tr>
<td>ROPC</td>
<td>-0.030</td>
</tr>
<tr>
<td>ROPR</td>
<td>0.191</td>
</tr>
<tr>
<td>ST interference time</td>
<td>-0.113</td>
</tr>
<tr>
<td>MWCST, achieved categories</td>
<td>-0.097</td>
</tr>
<tr>
<td>MWCST, perseverative errors</td>
<td>-0.146</td>
</tr>
<tr>
<td>n-2 back verbal</td>
<td><strong>0.809</strong></td>
</tr>
<tr>
<td>n-2 back visual</td>
<td><strong>0.793</strong></td>
</tr>
<tr>
<td>n-2 back spatial</td>
<td><strong>0.796</strong></td>
</tr>
<tr>
<td>Eigenvalues</td>
<td>4.983</td>
</tr>
<tr>
<td>Variance proportion</td>
<td>0.415</td>
</tr>
</tbody>
</table>

Note: RIR, Rey’s 15-Word Immediate Recall; RDR, Rey’s 15-Word Delayed Recall; PM47, Raven’s Progressive Matrices’ 47; TMT, Trail Making Test; ROPC, Rey-Osterrieth complex picture; ROPR, delayed recall of Rey-Osterrieth complex picture; ST, Stroop Test; MWCST, Modified Wisconsin Card Sorting Test. Variables fitted in factors are indicated in bold.
The first factor, which represents the “working memory” domain, accounted for 41.5% of total score variance and was composed by the n-2 back verbal, spatial, and visual tasks; the second factor, which represents the “verbal memory” domain, accounted for 13.8% of score variance and was composed by RIR and RDR; the third factor, which represents the executive functioning domain, accounted for 11.6% of total score variance and was composed by TMT ratio score, MWCST-achieved categories, and MWCST perseverative errors; and finally, the fourth factor was a residual domain that accounted for 10.5% of total score variance and was composed by heterogeneous tests measuring logical reasoning (PM47), visual perception/constructural praxis (ROPC), and attentional control and inhibition (ST interference time). It was called for convenience “visuospatial abilities” because all the 3 tests included required visuospatial abilities to complete them. Univariate correlation analyses were then computed between the 4 neuropsychological domains and the diffusivity and volume scores previously found to differentiate patients and controls.

Results of the correlation analyses are indicated in table 5. Correlation analyses showed that working memory domain was significantly and negatively correlated to MD score in right thalamus, right hippocampus, and left accumbens, with trend-level (P < .1) relationships observed with MD of the left hippocampus and with volume of the left ventricle. No other significant or trend-level relationships were observed between other neuropsychological domains and MD or volume values in the schizophrenic group.

**Discussion**

Combining volumetry and microstructure (DTI-MD) analyses of subcortical areas, we found significant increased MD values in thalami and hippocampi of both sides and in left accumbens. Volumetric changes, in the only direction of hypertrophy, were detectable in the left accumbens, and no subcortical atrophy was found. Furthermore, left lateral ventricle was enlarged in our sample of patients with diagnosis of schizophrenia without global cognitive impairment. The significant correlations between working memory domain and the MD values in right thalamus, right hippocampus, and left accumbens confirm the functional significance in terms of neuropsychological performance of the altered subcortical microstructure.

Here, we discuss how these findings point toward a model that emphasizes the presence of subtle, and potentially precocious, subcortical microstructural abnormalities in schizophrenia and their link with performances of high cognitive load level—such as
working memory—in patients with diagnosis of schizophrenia.

**Volume and Microstructural Abnormalities in Patients With Schizophrenia**

Result of increased MD in thalami and hippocampi in absence of significant subcortical volume atrophy suggests the presence, even in patients with diagnosis of schizophrenia with unimpaired global cognition, of subtle abnormalities only at a microstructural level in these areas. This is in line with neuroimaging and postmortem studies that have described microstructural and histological abnormalities in the thalamus and in the hippocampal formation in schizophrenia. To our knowledge, there are only 2 previous neuroimaging studies, focusing selectively on hippocampus and thalamus in patients with schizophrenia, suggesting that, in absence of morphological abnormalities, these 2 subcortical areas present microstructural abnormalities, ie, interval voxel coherences decrease and apparent diffusion coefficient increases, respectively. However, the anatomical areas investigated in these 2 studies were limited to one subcortical structure for each report, and the fine neuropsychological level was not considered. Thus, here, we provide the first evidence, to our knowledge, indicating that there are some subcortical areas that present microstructural abnormalities in absence of volume reduction in a large sample of patients with schizophrenia.

Our MD findings are also consistent with postmortem studies on schizophrenia, which have described a reduced total number of neurons and decreased volume in the thalamus (eg, ventral lateral posterior nucleus; medio-dorsal and anterior nucleus). As for the hippocampus, the abnormalities include a decrease in area or whole volume of the hippocampus, a decrease in the number of hippocampal neurons, reduced neuronal size and density, and pyramidal cell disarray. Interestingly, increased MD may be considered as a marker of a reduced number of cells and increased extracellular space. Therefore, we speculate that our findings of increased hippocampal and thalamic MD may reflect cell loss and impaired structural integrity in such subcortical regions in schizophrenia.

Consistently, thalamus and hippocampus are thought to play a pivotal role in the physiopathology of schizophrenia. As for the thalamus, our finding fits very well with model of thalamocortical dysfunction as a key component of etiopathological mechanism underling cognitive and also clinical symptoms in schizophrenia. Indeed, disruptions in thalamic circuitries such as the cortico-thalamic-thalamo-cortical as well as the corticocerebellar-thalamo-cortical networks have been proposed, by Andreasen et al., to lead to cognitive dysmetria, accounting for deficits in managing and performing information-processing tasks. However, previous studies used to build this model included subjects who were not selected for cognitive level and used thalamic volume or metabolism as an index of structural-functional abnormality. Interestingly, in our patient sample without global cognitive deterioration or dementia, we found that only a fine index of thalamic gray matter microstructure, such as MD, was abnormal, and this modification was negatively associated with working memory performance, which overlaps those abilities in managing, coordinating, and performing information-processing tasks described by Andreasen et al.

Hippocampus, as well as the thalamus, has been considered to be involved in the pathogenesis of schizophrenia, on the basis of evidence from neuroimaging, animal model, and histological studies. Indeed, because hippocampus has been described as a pivotal anatomical component of the aberrant schizophrenia functional connectivity, we can speculate that the altered microstructure in the hippocampi highlighted in our study may lead to disturbed information flow between the limbic circuit and the prefrontal cortex. This hypothesis is substantiated by a recent animal study that suggests that the dysfunction of information processing observed in patients with diagnosis of schizophrenia arises in part from a disturbed dopaminergic regulation among the hippocampus, accumbens, and prefrontal cortex. Consistently, we have found both increased MD and volume in the left accumbens in our sample of patients with diagnosis of schizophrenia. The increased volume in the accumbens is in line with postmortem studies in schizophrenia and is thought to reflect a decrease in naturally occurring cell death following prenatal cortical neurodevelopmental disturbances. Thus, the results of our study also provide the first evidence, to our knowledge, of altered accumbal MD in schizophrenia, which gives further support to the hypothesis proposing an important role for this structure in schizophrenia pathophysiology.

Taken all together, our DTI and volumetry results indicate that it is important to investigate in schizophrenia the presence of subtle microstructural changes localized in subcortical areas, such as thalami, hippocampi, and accumbens, which have been understood as key targets of pathophysiological processes in this disorder. Because thalamic volume reduction was found in some, but not all, studies in schizophrenia and hippocampal volume may depend on the psychosis diagnosis and stage, as well as on the cognitive level, it seems that altered subcortical microstructure, rather than the volumetry, may be a more consistent hallmark of subcortical pathology in schizophrenia.

Finally, we found increased left lateral ventricle volume in our schizophrenic group. Left-lateralized ventricular enlargement is one of the earliest and more consistent reported abnormalities in schizophrenia.
Left ventricular enlargement is commonly thought to reflect diffuse brain atrophy more prominent in the left side, but it may also be linked to volume reduction of adjacent subcortical regions (e.g., left thalamus). The greater enlargement seen on the left than on the right side may indicate that ventricular enlargement is, in part, a lateralized process, thereby supporting the hypothesis that schizophrenia is a disease of asymmetry. However, this topic is far from being defined with certainty and warrants further investigation particularly in subcortical areas, as indicated by the lack of statistical significance in analyses performed with scores obtained subtracting left-side brain subcortical structure values from right-side structure values.

**Neuropsychological Correlates of Subcortical Microstructural Damage in Patients With Diagnosis of Schizophrenia Without Global Cognitive Impairment**

In order to identify the functional meaning, in terms of neuropsychological performance, of subcortical abnormalities in the schizophrenic group, we analyzed the relationships between MD and volume values found to be different between case-control groups and neuropsychological domains. We found that increased working memory performance was related to microstructure integrity, measured in terms of MD, in the right thalamus, right hippocampus, and left accumbens, with also some relationships at a trend level with left hippocampus and volume of left ventricle.

These findings fit very well with the hypotheses that consider subcortical structures to play a crucial role in working memory performance, in view of their connections with the brain cortices, not only at the prefrontal level but also at the parietal and temporal levels. Indeed, research on schizophrenia has identified a distributed network of cortical and subcortical regions involved in working memory, particularly emphasizing the role of the frontostriatal dopaminergic circuits. For instance, attention, an important component of working memory, is thought to be subserved by a mostly right-lateralized frontal, parietal, and thalamic network. Thus, our result of working memory domain as mainly related to right-lateralized thalamic and hippocampal microstructural damage provides additional evidence on the role of the right hemisphere in cognition in schizophrenia, as efficaciously summarized by Mitchell and Crow. On the other hand, results indicating the trend-level relationships between working memory performance and enlarged left ventricle and left hippocampal MD seem to suggest a bilateral involvement of brain structures and functions in schizophrenia.

Furthermore, our data indicate that this neuropsychological corticosubcortical model emerging from previous research in schizophrenia might be further developed. More subtle microstructural subcortical changes seem to play a key role in working memory function. Our findings suggest that this ability of high cognitive load level is subserved by a distributed cortical-subcortical network that includes structures such as thalamus, hippocampus, and accumbens that are altered in their microstructures in patients with diagnosis of schizophrenia. Consequently, reduced working memory performances in schizophrenia are not necessarily related to subcortical volumetric abnormalities but rather to more subtle microstructural alterations.

**Strengths and Limitations of the Study and Conclusions**

To the best of our knowledge, this is the first study applying a combined volumetry and DTI method on subcortical nuclei in the same sample of patients with diagnosis of schizophrenia. Also, we provide the first evidence on the neuropsychological correlates of microstructural abnormalities in the subcortical nuclei in schizophrenia.

Nevertheless, some limitations need to be drawn for the generalization of the results. Firstly, all patients with diagnosis of schizophrenia in our study received cumulative doses of atypical antipsychotics, whose influence on the results must be taken into consideration. However, performing a series of univariate correlation analyses, we failed to find any relationship between antipsychotic dosages and subcortical MD or volume values in our sample, with the exception of a weak and not significant correlation with MD score in the left hippocampus (data available upon request). Therefore, we exclude a relevant confounding effect of medication on our results. However, follow-up studies in larger cohorts of drug-naïve, first-episode schizophrenic subjects are required to explore the full potential use of combining DTI with volumetry on subcortical nuclei.

Secondly, further studies are necessary to clarify the link between subcortical, microstructural, and volumetric abnormalities and clinical symptoms in schizophrenia, which was not among the aims of the present study. Thirdly, we chose to include in our study only schizophrenic patients without global cognitive impairment as assessed by MMSE on the basis of previous evidence that global cognitive impairment may act as a potential confounding variable on volumes of subcortical brain structures. However, it could be argued that our MMSE cutoff score could have excluded patients who were most impaired in other cognitive domains. To test this hypothesis, we performed a series of univariate correlation analyses and found only weak and nonsignificant correlations between the MMSE score and the neuropsychological domains: working memory \((r = 0.252, P = 0.0953)\), verbal memory \((r = 0.275, P = 0.0670)\), executive function \((r = 0.069, P = 0.6564)\), and visuospatial abilities \((r = 0.291, P = 0.0517)\). Thus, it is not very probable that
this limitation may compromise the generalization of our results.

In conclusion, this study was able to highlight subtle microstructural damage in subcortical areas present even in absence of detectable volumetric changes in patients with diagnosis of schizophrenia without global cognitive deterioration. The microstructural alterations in subcortical regions, ie, hippocampus, thalamus, and left accumbens, play a pivotal role in working memory performances.

Finally, our findings indicate that MD is a more sensitive marker of brain tissue deficits than signal intensity variations measured in T1-weighted imaging data, consistently with previous reports. This is intriguing, also considering the great variability across volumetric studies in schizophrenia. Thus, DTI appears to be an invaluable tool to investigate subcortical pathology in schizophrenia, greatly enhancing the ability to detect subtle brain changes in this complex disorder.

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References


Subcortical Microstructure and Volumetry in Schizophrenia


32. Knight JA, Kaplan E. The Handbook of Rey-Osterrieth Complex Figure Usage: Clinical and Research Applications. Odessa, FL: Psychological Assessment Resources; 2003.


57. Young KA, Manaye KF, Liang C, Hicks PB, German DC. Reduced number of mediodorsal and anterior thalamic neurons in schizophrenia. *Biol Psychiatry.* 2000;47:944–953.


69. Gray JA. Integrating schizophrenia. *Schizophr Bull.* 1998;24:249–266.


