

# Structural Abnormalities in Frontal, Temporal, and Limbic Regions and Interconnecting White Matter Tracts in Schizophrenic Patients With Prominent Negative Symptoms

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**Objective:** Imaging studies of schizophrenia have repeatedly demonstrated global abnormalities of cerebral and ventricular volumes. However, pathological changes at more local levels of brain organization have not yet been so clearly characterized because of the few brain regions of interest heretofore included in morphometric analyses as well as heterogeneity of patient samples.

**Method:** Dual echo magnetic resonance imaging (MRI) data were acquired at 1.5 T from 27 right-handed patients who met DSM-IV criteria for schizophrenia with enduring negative symptoms and from 27 healthy comparison subjects. Between-group differences in gray and white matter volume were estimated at each intracerebral voxel after registration of the images in standard space. The relationship between clinical symptom scores and brain structure was also examined within the patient group. Spatial statistics and permutation tests were used for inference.

**Results:** Significant deficits of gray matter volume in the patient group were found at

three main locations: 1) the left superior temporal gyrus and insular cortex, 2) the left medial temporal lobe (including the parahippocampal gyrus and hippocampus), and 3) the anterior cingulate and medial frontal gyri. The volume of these three regions combined was 14% lower in the patients relative to the comparison subjects. White matter deficits were found in similar locations in the left temporal lobe and extended into the left frontal lobe. The patient group showed a relative excess of gray matter volume in the basal ganglia. Within the patient group, basal ganglia gray matter volume was positively correlated with positive symptom scores.

**Conclusions:** Anatomical abnormalities in these schizophrenic patients with marked negative symptoms were most evident in left hemispheric neocortical and limbic regions and related white matter tracts. These data are compatible with models that depict schizophrenia as a suprapregional disorder of multiple, distributed brain regions and the axonal connections between them.

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It has now been more than 20 years since the first neuroimaging study of anatomical abnormalities associated with schizophrenia was published (1). Since then, two findings in particular have been well replicated: diffuse enlargement of lateral and third ventricles by approximately 10% and diffuse reduction in cortical gray matter volume by approximately 3%–4% (2–4). Many researchers have adopted a region-of-interest approach to morphometry, whereby the areas or volumes of a limited number of brain regions are estimated by manual delineation of their boundaries on imaging data. In this way, gray matter deficits, and to a lesser extent white matter deficits, have been reported for frontal and temporal lobes. Gray matter deficits have been reported especially for medial temporal lobe structures such as the hippocampus and amygdala. Yet efforts to characterize pathological change with greater regional specificity have so far failed to identify a single focus of disproportionate volume loss (see Wright et

al. [5] for a meta-analysis of volumetric magnetic resonance imaging [MRI] studies in schizophrenia).

One likely reason for the inconclusiveness of this literature is that pathological changes in brain structure due to schizophrenia are not limited to one or a few clearly delimited brain regions. There is indeed considerable evidence to suggest that pathological change in patients with schizophrenia may be expressed at the level of spatially distributed networks that subsume multiple, densely interconnected cortical and subcortical regions (6–10). Another possible reason is that schizophrenia is a heterogeneous collection of syndromes that may differ in many ways, including the profile of symptoms determined by variable anatomical abnormalities.

In this study, we attempt to address both these issues. First, we studied a clinically homogeneous group of schizophrenic patients with primary and enduring negative symptoms. Second, we used automated morphomet-

ric methods that did not restrict attention to previously defined regions of interest but instead estimated gray and white matter deficits that were related to diagnosis or behavioral measures such as clinical symptom scores at each voxel of the images after registration in standard space.

## Method

### Subjects

Twenty-seven right-handed patients with schizophrenia and 27 healthy right-handed comparison subjects were studied. The patients were recruited from the Maudsley and Bethlem Royal Hospitals, London. Comparison subjects were recruited by advertisements in local newspapers and from hospital staff. All subjects satisfied the following criteria for inclusion in the study: no history of alcohol or drug dependence, no history of head injury causing loss of consciousness for 1 hour or more, and no history of neurologic or systemic illness. Comparison subjects satisfied the additional criterion of having no DSM-IV axis I disorder. There were no significant between-group differences in age, sex, or parental social class (defined by the occupation of head of household at time of birth [11]). Mean premorbid IQs for both groups as estimated by the National Adult Reading Test (12) were in the normal range, but the comparison subjects had a slightly, albeit significantly, higher mean IQ than the patients. These and other details are given in Table 1.

After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the Ethical Committee (Research) of the Bethlem Royal and Maudsley National Health Service Trust.

### Clinical Assessments

Diagnoses were made following a clinical interview and case-note review. The patients met DSM-IV criteria for schizophrenia and were in a stable phase of their illness. They were also required to meet criteria for the deficit syndrome of schizophrenia so that only patients with primary and enduring negative symptoms were included (13). One rater (T.S.) administered the Positive and Negative Syndrome Scale (14) on the day of scanning to measure psychopathology during the week before assessment.

### Structural MR Image Acquisition

All subjects were scanned with a GE Signa 1.5-T system (GE Medical Systems, Milwaukee) at the Maudsley Hospital, London. A preliminary localizing scan in the coronal plane was used to identify anterior and posterior commissures and to prescribe acquisition of a dual echo fast spin echo dataset in an axial plane parallel to the intercommissural line. Contiguous, interleaved proton density- and T<sub>2</sub>-weighted images, each 3-mm thick, were obtained to provide whole brain coverage. Repetition time (TR) was 4000 msec and echo times (TE) were 20 and 85 msec with an 8-echo train length. The matrix size was 256 × 192 collected from a rectangular field of view of 22 cm × 16.5 cm, giving an in-plane resolution of 0.859 mm. The total acquisition time was 10 minutes and 12 seconds.

### Structural MRI Data Analysis

The methods used for segmentation and registration of each fast spin echo dataset have been described in detail elsewhere (15, 16) but were briefly as follows. Voxels representing extracerebral tissue were automatically identified and set to zero by using a linear scale space set of features obtained from derivatives of the gaussian kernel (15). Manual editing of the segmented images was necessary only to remove brainstem and cerebellum from the cerebral hemispheres and diencephalon. The probabil-

**TABLE 1. Demographic and Clinical Characteristics of Schizophrenic Patients With Prominent Negative Symptoms and Healthy Comparison Subjects**

| Characteristic                       | Patients (N=27) |           | Comparison Subjects (N=27) |           |
|--------------------------------------|-----------------|-----------|----------------------------|-----------|
|                                      | N               | %         | N                          | %         |
| Gender                               |                 |           |                            |           |
| Male                                 | 26              | 96        | 25                         | 93        |
| Female                               | 1               | 4         | 2                          | 7         |
| Social class 1–3                     | 20              | 74        | 22                         | 81        |
|                                      | <i>Mean</i>     | <i>SD</i> | <i>Mean</i>                | <i>SD</i> |
| Age (years)                          | 34.9            | 7.6       | 32.2                       | 6.7       |
| Education (years) <sup>a</sup>       | 12.3            | 2.3       | 14.2                       | 2.7       |
| Premorbid IQ <sup>b</sup>            | 102.3           | 13.5      | 109.6                      | 10.8      |
| Global Assessment Scale score        | 42.5            | 13.8      | —                          | —         |
| Positive and Negative Syndrome Scale |                 |           |                            |           |
| Total score                          | 74.5            | 11.2      | —                          | —         |
| Positive score                       | 14.7            | 4.8       | —                          | —         |
| Negative score                       | 25.0            | 5.9       | —                          | —         |
| Global score                         | 34.7            | 5.9       | —                          | —         |
| Duration of illness (years)          | 13.9            | 6.6       | —                          | —         |

<sup>a</sup> Significant difference between groups ( $t=2.7$ ,  $df=52$ ,  $p<0.007$ ).

<sup>b</sup> Significant difference between groups ( $t=2.1$ ,  $df=52$ ,  $p<0.04$ ).

ity of each intracerebral voxel belonging to each of four possible tissue classes (gray matter, white matter, CSF or dura/vasculature) was then estimated by a modified fuzzy clustering algorithm (16). This algorithm was applied by means of a “sliding window” to the images so that classification was adaptive to local variation in tissue contrast due to radiofrequency or static field inhomogeneity (see reference 16 for details). On the basis of prior results, we assumed that the resulting probabilities of tissue class membership could be equated with the proportional volumes of each tissue class in the often heterogeneous volume of tissue represented by each voxel (17). So, for example, if the probability of gray matter class membership was 0.80 for a given voxel, then it was assumed that 80% of the tissue represented by that voxel was gray matter. Given the voxel size (2.2 mm<sup>3</sup>), it was straightforward to estimate the volume in milliliters of gray matter, or any other tissue class, at each voxel. Summing these voxel tissue class volumes over all intracerebral voxels yielded global tissue class volumes.

To allow estimation of between-group structural differences and structure-psychopathology associations at each intracerebral voxel, the proton density-weighted images from each fast spin echo dataset were first coregistered with a template image in the standard space of Talairach and Tournoux (18) by an affine transformation, which we implemented using the Fletcher-Davidon-Powell algorithm (19, 20). (The template image was constructed by registering each of six images acquired from a subset of the comparison subjects in this study [three male, three female] in standard space by an affine transformation and then averaging these images.) The affine transformation matrix that mapped each subject's proton density-weighted image onto this template image was then applied identically to each of that subject's four tissue class probability maps to register them in standard space at the same voxel size as the original acquisition.

Between-group differences in gray matter volume were estimated by fitting the following analysis of covariance model at each intracerebral voxel in standard space (equation 1):

$$G_{i,j,k} = \mu_i + \beta_1 \Delta_k + \beta_2 \text{Sex}_{j,k} + \varepsilon_{i,j,k}$$

Here,  $G_{i,j,k}$  denotes the gray matter volume at the  $i$ th voxel in the  $j$ th member of the  $k$ th group ( $j=1, 2, 3, \dots, 27$ ;  $k=1, 2$ );  $\mu_i$  denotes

**TABLE 2. Whole Brain, Gray Matter, White Matter, CSF, and Gray and White “Deficit” Region Volumes of Schizophrenic Patients With Prominent Negative Symptoms and Healthy Comparison Subjects**

| Cerebral Region                            | Volume (ml)     |     |           |                            |     |           | Analysis  |         |                      |
|--|-----------------|-----|-----------|----------------------------|-----|-----------|-----------|---------|----------------------|
|  | Patients (N=27) |     |           | Comparison Subjects (N=27) |     |           | t (df=52) | p       | 95% CI of Difference |
|  | Mean            | SD  | 95% CI    | Mean                       | SD  | 95% CI    |           |         |                      |
| Whole brain                                | 1298            | 122 | 1242–1352 | 1358                       | 136 | 1297–1404 | 1.7       | 0.09    | –10.0 to 130.0       |
| White matter                               | 586             | 67  | 558–621   | 624                        | 72  | 592–652   | 1.9       | 0.05    | –0.1 to 76.0         |
| Gray matter                                | 509             | 55  | 481–524   | 538                        | 68  | 507–559   | 1.7       | 0.09    | –4.6 to 63.0         |
| CSF  | 161             | 34  | 142–171   | 150                        | 27  | 137–158   | –1.3      | 0.18    | –28.0 to 5.4         |
| Gray matter “deficit” region <sup>a</sup>  | 4.3             | 0.3 | 4.2–4.5   | 5.0                        | 0.2 | 4.9–5.1   | 8.8       | <0.0005 | 0.5 to 0.8           |
| White matter “deficit” region <sup>b</sup> | 7.8             | 0.8 | 7.4–8.1   | 8.9                        | 0.3 | 8.7–9.0   | 7.0       | <0.0005 | 0.8 to 1.4           |

<sup>a</sup> Three voxel clusters in which relative gray matter volume deficits were seen in the patient group. See text for description of anatomical areas in which the voxel clusters extended.

<sup>b</sup> One voxel cluster in which relative white matter volume deficits were seen in the patient group. See text for description of the anatomical area in which the voxel cluster extended.

the overall mean at the *i*th voxel;  $\mu_i + \beta_1 \Delta_k$  is the mean gray matter volume for the *k*th group;  $Sex_{j,k}$  denotes the sex of the *j*th member of the *k*th group; and  $\epsilon_{i,j,k}$  is a residual. The same model was applied to estimate between-group differences in white matter volume.

The relationship between gray and white matter volume and psychopathology within the patient group was estimated by fitting the following multiple regression model at each intracerebral voxel in standard space for each tissue class separately (equation 2):

$$G_{i,m} = \mu_i + \beta_1 P_m + \beta_2 Sex_m + \epsilon_{i,m}$$

Here,  $P_m$  denotes psychopathology score for the *m*th subject ( $m=1, 2, 3, \dots, 27$ ), and the other abbreviations are as before. The psychopathology scores used were the individual total positive symptom scores and, in a separate analysis, the total negative symptom scores from the Positive and Negative Syndrome Scale.

In both cases, the null hypothesis (i.e.,  $\beta_1=0$ ) was tested by permutation at cluster level, as described in detail elsewhere (21); the procedure is briefly described for the multiple regression analysis (equation 2). A map of the standardized coefficient  $\beta_1/SE(\beta_1)$  at each voxel was thresholded such that if  $\beta_1/SE(\beta_1) > 2$ , the voxel value was set to  $\beta_1/SE(\beta_1) - 2$ ; otherwise, the voxel value was set to zero. This procedure generates a set of suprathreshold voxel clusters in three dimensions, each of which can be described in terms of its mass or the sum of suprathreshold voxel statistics it comprises. The mass of each cluster was tested against a null distribution ascertained by repeatedly randomly permuting the vector that coded psychopathology scores in equation 2, refitting the model and estimating standardized coefficients at each voxel, and then thresholding the permuted coefficient maps to generate a set of three-dimensional suprathreshold voxel clusters under the null hypothesis. The rationale for this nonparametric mode of inference is that test statistics for image analysis that incorporate spatial information, such as three-dimensional cluster mass, are generally more powerful than other possible test statistics, such as  $\beta_1/SE(\beta_1)$ , which are informed only by data at a single voxel. Yet theoretical approximations to the null distribution of spatial statistics estimated in imaging data may be overconservative or intractable (21, 22). The principles of permutation testing were first established by Fisher (23) and Pitman (24) in the 1930s but have found increasingly wide application with the recent advent of powerful microprocessors (see Edgington [25], Good [26], and Manly [27] for good introductory texts).

## Results

### Global Brain and Tissue Class Volumes

Global volumes for whole brain and each of the three main tissue classes (gray matter, white matter, and CSF) are shown in Table 2. Whole brain volume in the patient group was 4% smaller than in the comparison group, although this difference was not significant. White and gray matter volumes in the patient group were also 5%–6% smaller; this difference was on the threshold of statistical significance for white matter but not significant for gray matter. CSF volume was 7% higher in the patient group, but again this difference was not significant.

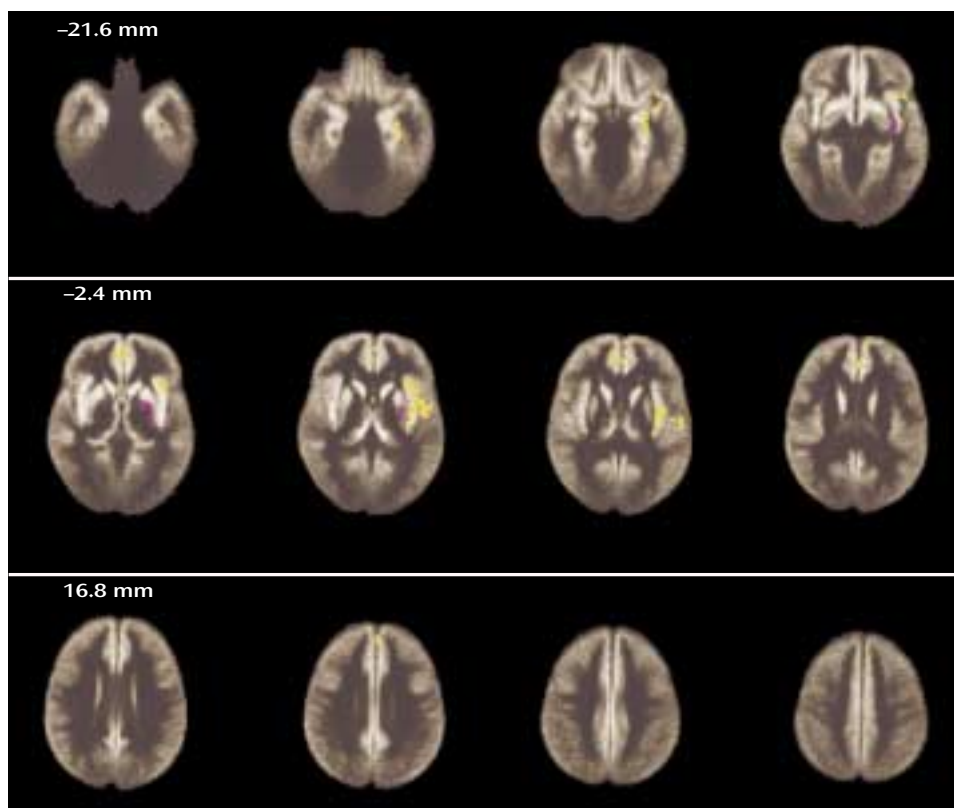
### Localized Between-Group Differences in Gray Matter Volume

A significant difference between the schizophrenic and comparison groups in gray matter volume was identified at four spatially extensive three-dimensional voxel clusters. Three clusters of voxels showed lower gray matter volume in the patient group relative to the comparison group: 1) a perisylvian cluster, extending from the left superior temporal gyrus (approximate Brodmann’s area 22) anteriorly to the insula and the opercular part of the inferior frontal gyrus (Brodmann’s area 44); 2) a medial frontal cluster, extending bilaterally from the medial frontal gyrus (Brodmann’s area 10) to the anterior cingulate gyrus (Brodmann’s area 32); and 3) a medial temporal cluster, extending from the parahippocampal gyrus to the hippocampus and amygdala. One subcortical cluster that included the putamen, nucleus accumbens, and globus pallidus showed a relative excess of gray matter volume in the patient group. The perisylvian, medial temporal, and subcortical clusters were all located in the left hemisphere.

To identify these differences, a total of 502 suprathreshold three-dimensional clusters were tested by permutation, with cluster-wise probability of type I error set at  $p=0.001$ . At this size of test, we expect less than one false positive cluster over the search volume. The mean between-group difference in gray matter volume for the combined deficit regions was 14%, which was highly significant (Table 2). Further details, including Talairach coordinates for

**TABLE 3. Gray and White Matter Regional Differences Between Schizophrenic Patients With Prominent Negative Symptoms (N=27) and Healthy Comparison Subjects (N=27)**

| Tissue Class and Relative Difference Region   | Brodmann's Area | Side      | Number of Voxels in Cluster | Location of Cluster Center |      |      |
|---|-----------------|-----------|-----------------------------|----------------------------|------|------|
|   |                 |           |                             | x                          | y    | z    |
| <b>Gray matter</b>  |                 |           |                             |                            |      |      |
| <b>Deficits</b>   |                 |           |                             |                            |      |      |
| Perisylvian region  | 22/44           | Left      | 1,979                       | -38                        | -4   | -7   |
| Medial frontal lobe/anterior cingulate  | 10              | Bilateral | 1,022                       | -0.5                       | 46   | 1    |
| Parahippocampal gyrus   | 35              | Left      | 573                         | -29                        | -6   | -26  |
| Excess: putamen/globus pallidus   | —               | Left      | 559                         | -26                        | -3.6 | -12  |
| <b>White matter deficit: region involving the uncinate fasciculus and extending into the inferior longitudinal fasciculus; also involving the anterior limb of the internal capsule and extending into the corpus callosum and frontal lobe</b> |                 |           |                             |                            |      |      |
|   | —               | Left      | 5,150                       | -25.8                      | 0.0  | -1.4 |

**FIGURE 1. Gray Matter Volume Differences Between Schizophrenic Patients With Prominent Negative Symptoms (N=27) and Healthy Comparison Subjects (N=27)<sup>a</sup>**

<sup>a</sup> Yellow regions denote areas of gray matter deficits in the patient group relative to the comparison group; purple regions denote areas of greater gray matter volume in the patient group relative to the comparison group. The left side of each panel represents the right side of the brain; the z coordinate for each axial slice in the standard space of Talairach and Tournoux (18) is given in millimeters. Cluster-wise probability of type I error:  $p=0.001$ , meaning less than one false positive test is expected over the whole map.

each cluster center, are given in Table 3. A map showing colored areas of significant between-group difference superimposed on the mean gray matter template image is shown in Figure 1.

#### **Localized Between-Group Differences in White Matter Volume**

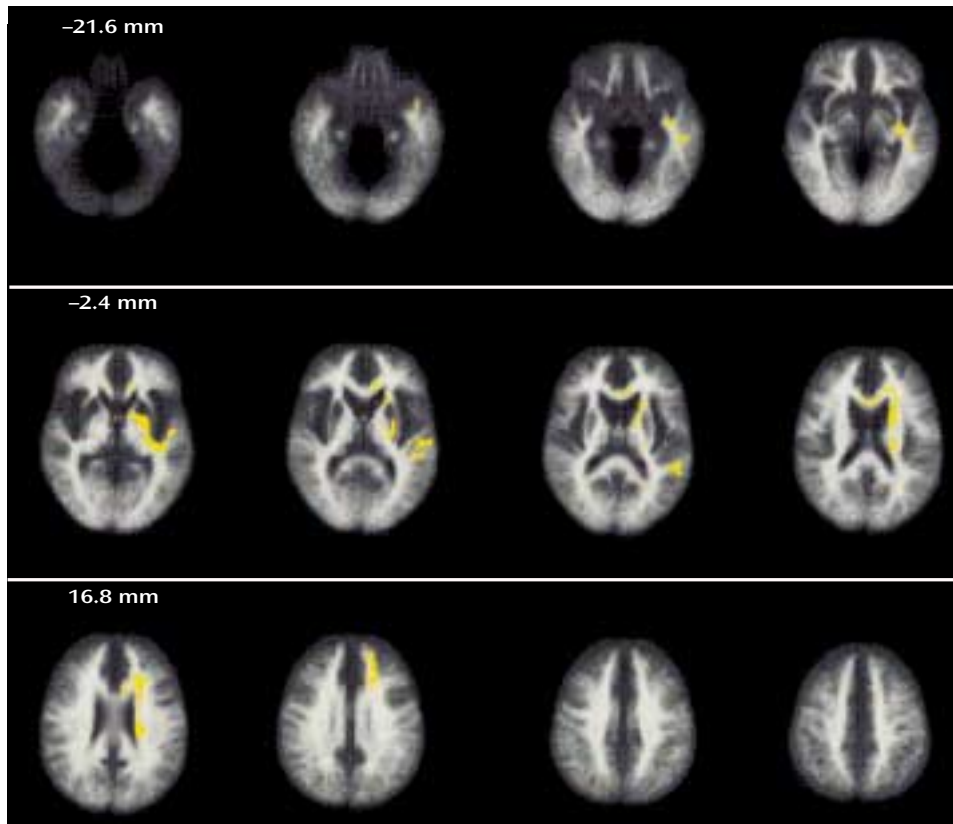
A significant difference in white matter volume between the schizophrenic and comparison groups was identified at one extensive three-dimensional cluster located in the

left hemisphere. This cluster involved the uncinate fasciculus and extended posteriorly into the inferior longitudinal fasciculus and into the inferior parietal lobe on the left side. It also involved the anterior limb of the internal capsule and extended superiorly into the superior longitudinal fasciculus, anterior corpus callosum, and the frontal lobe.

The number of three-dimensional clusters tested by permutation was 470, with cluster-wise probability of type I error set at  $p=0.001$ . We expected to observe less than one



FIGURE 2. White Matter Volume Differences Between Schizophrenic Patients With Prominent Negative Symptoms (N=27) and Healthy Comparison Subjects (N=27)<sup>a</sup>



<sup>a</sup> Yellow regions denote areas of white matter deficits in the patient group relative to the comparison group. The left side of each panel represents the right side of the brain; the z coordinate for each axial slice in the standard space of Talairach and Tournoux (18) is given in millimeters. Cluster-wise probability of type I error:  $p=0.001$ , meaning less than one false positive test is expected over the whole map.

false positive cluster over the search volume at this statistical threshold. The size of the cluster and Talairach coordinates for the center of the cluster are given in Table 3. The total volume of white matter represented by the voxels that made up this cluster is shown in Table 2. The between-group difference in white matter volume for this region was 13% (Table 2). A map showing colored areas of significant white matter deficit superimposed on the white matter template image is shown in Figure 2.

### ***The Relationship Between Clinical Symptoms and Brain Structure***

We found no significant association between gray or white matter volume and negative symptoms in the patient group. There was a significant association between the positive symptom score and gray matter volume at two large bilateral clusters centered on the basal ganglia (caudate nucleus, putamen, and globus pallidus). At both these sites, an excess of gray matter volume was predicted by a higher positive symptom score. The left-sided cluster extended into the orbitofrontal lobe, and the right-sided cluster extended into the insula and superior temporal gyrus. There was also a significant association between white matter volume and positive symptom score in two

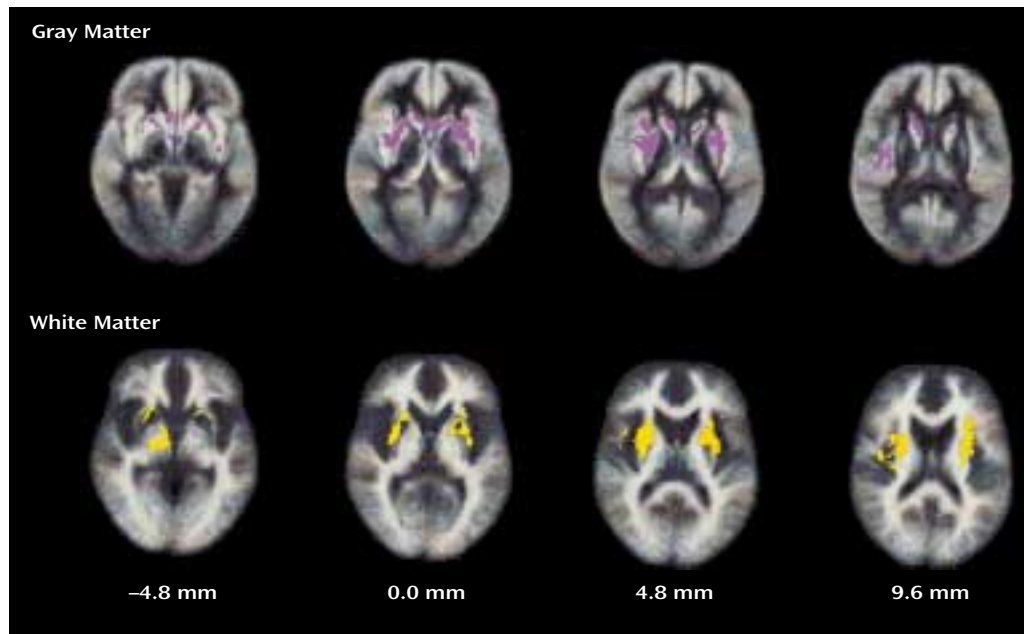
bilateral clusters adjacent to the basal ganglia that showed a deficit of white matter was associated with a high positive symptom score.

The number of clusters tested by permutation in this analysis was 700, and with the cluster-wise probability of type I error set at  $p=0.001$  we expected to find less than one false positive cluster over the search volume. The size and location of the clusters are shown in Table 3, and a map showing colored areas of significant association is shown in Figure 3.

## **Discussion**

One of the main findings of this study was that significant deficits of gray matter volume in a symptomatically homogeneous group of patients with schizophrenia could be localized to three brain regions by using an almost entirely automated cluster level analysis of dual echo MR images. The regions of deficit included the anterior cingulate and middle frontal cortex, the left medial temporal lobe (including the hippocampus and parahippocampal gyrus), and, most extensively, the left superior temporal gyrus, inferior frontal gyrus, and insular cortex. There was

**FIGURE 3. Association Between Positive Symptom Scores and Tissue Class Volume in Schizophrenic Patients With Prominent Negative Symptoms (N=27)<sup>a</sup>**



<sup>b</sup> Purple regions denote areas in which greater gray matter volume is predicted by a high score on a positive symptom rating scale; yellow regions denote areas in which lower white matter volume is predicted by a high positive symptom score. The left side of each panel represents the right side of the brain; the z coordinate for each axial slice in the standard space of Talairach and Tournoux (18) is given in millimeters.

also one area in which there was a relative excess of gray matter volume in the patient group, which was in the region of the globus pallidus and putamen on the left side.

Previous structural MRI studies have frequently adopted the hippocampus and parahippocampal gyrus as regions of interest, and volumetric reduction of both regions has been repeatedly demonstrated (for a review see the meta-analysis by Nelson et al. [28]). Reduction of the neuronal integrity marker *N*-acetylaspartate, suggesting neuronal loss, has also been reported in the medial temporal lobe, and these findings have been more marked on the left (29–31). There have also been reports of a reduction in the volume of the superior temporal gyrus in schizophrenia (32–36), which is consistent with our findings. Although our analysis found gray matter deficits only in the left temporal lobe, at a less stringent significance level ( $p=0.005$ ), we also detected deficits in comparable areas in the superior temporal gyrus on the right side (Talairach coordinates of the cluster center:  $x=49$ ,  $y=9$ ,  $z=-14$ ).

There is not as much prior evidence for abnormalities of the insular cortex in schizophrenia. Perhaps because of its location, isolated lesions to the insula are rather uncommon. However, Shuren (37) reported speech initiation difficulties following left anterior insular infarction, and Dronkers et al. (38) suggested that lesions in the left insula produce deficits in the articulatory planning of speech. Cancelliere and Kertesz (39) reported deficits of emotional expression and comprehension in patients with insular lesions. These features of insular damage are also found in patients with schizophrenia, especially in patients with

marked negative symptoms of alogia and autism. Neuropathological evidence for insular abnormalities in schizophrenia is limited, but Jacob and Beckmann (40) have reported cytoarchitectonic abnormalities in the insular cortex. However, the insula has been neglected as a region of interest in previous MRI studies. It is possible that previously reported increases in size of the CSF-filled Sylvian fissure and temporal sulci may reflect some underlying loss of insular cortex volume (2, 41). But the clearest imaging evidence to date of gray matter loss in the insula has come from studies that have used voxel-based approaches to morphometry similar to our methods (10).

Similarly, very few previous imaging studies of brain structure in schizophrenia have adopted the medial frontal cortex as a region of interest. However, it has previously been suggested that abnormalities in the anterior cingulate and medial frontal lobe may be responsible for some of the negative or deficit features of schizophrenia. Disruption of a cingulate-striatal circuit causes a behavioral syndrome characterized by apathy, lack of drive, and perseveration (42). Liddle et al. (43) found a negative correlation between psychomotor poverty and cerebral blood flow in the anterior cingulate and medial frontal lobe. Dolan et al. (44) reported abnormal modulation of anterior cingulate activation by apomorphine in patients with schizophrenia. Macroscopic gray matter volume decrements in the medial frontal cortex, such as we have shown, are broadly compatible with these lesion and functional imaging studies as well as with neuropathological evidence for reduced medial frontal cortical thickness in pa-

tients with schizophrenia (45). It is interesting to note that Benes (45) found increased neuronal density in medial frontal areas, suggesting that reduced cortical thickness was due to impoverished dendritic arborization rather than neuronal depopulation. Since dendritic arborization is a microscopic marker for interneuronal connectivity, it is perhaps not surprising that medial frontal cortical changes in this group were associated with gray matter decrements in several anatomically connected cortical and subcortical regions.

It is known from studies of anatomical connectivity between homologous areas of nonhuman primate cortex that the brain regions we have identified as deficient in schizophrenia are normally linked to each other by dense and reciprocally afferent white matter tracts (46, 47). This suggests that we should consider pathological change in schizophrenia to be identified at the supraregional level of large-scale neurocognitive networks (48) rather than at the regional level of analysis preferred by most previous imaging and histological studies. The most salient piece of evidence in favor of such a network model of pathology from our data is the co-occurrence of white matter deficits in left hemispheric tracts that are known to incorporate axonal connections between the regions of gray matter deficit. These areas of white matter deficit included periventricular regions and might, in part, explain the (nonsignificantly) increased volume of CSF spaces in the patient group. In our view it is unlikely that this causal relation is reversed (i.e., that ventricular enlargement should have caused white matter deficit), since 1) that mechanism would imply raised intraventricular pressure—which has not been shown in schizophrenia—and 2) the areas of white matter deficit extended far from the lateral ventricles, reaching the immediately subcortical parts of frontal and temporal lobes.

There have been few previous histological or imaging studies of white matter in schizophrenia, despite the longstanding conjecture that a disorder of the association fibers may be implicated in psychosis (49). However, white matter deficits in frontal lobe regions of interest have been previously reported (50, 51), and the recently developed technology of diffusion weighted imaging has shown widespread abnormalities of white matter organization and orientation of fiber tracts in patients with schizophrenia (52). Neither these findings nor our own data allow us to be conclusive about the relative priority of gray and white matter deficits in schizophrenia. One could argue, within the general framework of the neurodevelopmental hypothesis, that the primary “lesion” is in the neuronal cell bodies of gray matter, and this causes abnormal axonal projection and maturation that leads to secondary changes in adult white matter structure. With almost equal plausibility, however, one could claim that the primary lesion affects axonal growth or synapse formation, and the structure of adult gray matter is abnormal because it has been deprived of the mutually trophic effects nor-

mally mediated by active synaptic connections between developing brain regions. These and other questions must await more etiologically or pathogenetically oriented studies.

Another important finding was an area of excess gray matter volume in the left basal ganglia. Similar findings have previously been reported following region-of-interest analysis of the caudate and putamen (53, 54) and generally are attributed to the hypertrophic effects of prolonged dopamine D<sub>2</sub> receptor antagonism by antipsychotic drugs. In our data, both bilateral enlargement of basal ganglia gray matter and a unilateral (right-sided) deficit of adjacent white matter were positively correlated with positive symptom scores. This relationship might reflect the tendency for patients with more severe positive symptoms to receive larger doses of antipsychotic medication, although this could not be confirmed in our study. Alternatively, these subcortical abnormalities may be a cause rather than an effect of positive symptoms.

Other areas of relative gray matter increase in relation to increased positive symptom scores identified in the patient group cannot be readily discounted as side effects of medication. Higher gray matter volume in the right superior temporal gyrus and insula, for example, has not previously been linked to antipsychotic drug exposure, but there is some functional imaging evidence to suggest relatively enhanced activation of these areas by auditory-verbal stimulation in patients with schizophrenia and a history of auditory-verbal hallucinations (55). Speculatively, these data are consistent with the hypothesis of Crow et al. (56) that a pathological process involving the left (normally dominant) perisylvian area, perhaps early in the course of development, may be partly compensated by a reorganization of contralaterally homologous areas, leading to abnormally increased right (normally nondominant) temporal cortical size and functional responsivity.

There are several methodological questions arising in consideration of these results, probably the most important of which concern the methods of computational morphometry we have used. To summarize the image processing “pipeline” used here: 1) we constructed a template image in Talairach space by spatially normalizing and averaging images from a subset (N=6) of the comparison subjects; 2) after locally adaptive and automated tissue classification in native space, we registered all images (N=54) with this template by an affine transformation; 3) finally, we tested for differences in tissue classification by permutation tests on spatial statistics. These procedures have been described and validated in detail elsewhere (1, 4, 16, 17). As we have noted, many of our results are corroborated by prior imaging studies of schizophrenia that used region-of-interest morphometry (although results of regional abnormality in the medial frontal cortex, insula, and white matter are difficult to cross-validate in this way, since these regions have not often been regarded a priori as interesting [5]). However, this sequence of image pro-

cessing operations does not represent a unique solution to the complex problem of extracting anatomical information from MRI datasets. In particular, we have used a relatively simple (affine) transformation to match images to a template. This linear transformation globally resizes and reorients the images relative to the template; it does not rescale voxel gray scale values. It is intended to minimize global size and orientation differences between images but not to eliminate more local differences. It is these residual (postregistration) differences that were comprehensively measured and tested for evidence of a main effect of diagnostic group. A fundamentally different analytic strategy is to use a higher-order or nonlinear transformation that aims to match images to a template so precisely that there are zero residual differences between images. In this case, the test statistic for anatomical disparity between groups must be the deformation applied at each voxel to bring it into perfect registration with the template rather than the residual difference in voxel values after a deliberately imperfect registration (see Thompson et al. [57] for an example of this approach and references to related work). Methods for computational morphometry are an actively developing focus of brain mapping research, and there is insufficient comparative data to allow a fully informed choice between these two rather different strategies—analysis of residual differences after linear transformation and analysis of the nonlinear transformations required to eliminate residual differences (22). We conclude that our methods are wholly reliable and valid insofar as we have evaluated them, but they do not represent the only possible solution to the problem they address.

There are a number of subsidiary issues to consider methodologically. First, the study group size was small. One implication of this is that some of the nonsignificant differences we have reported may be type II (false negative) errors. This seems particularly likely to be the reason for our failure to show significant between-group differences in global tissue and CSF volumes, which were of the same order as those previously reported as significant in a larger study (2). Second, the patient group was not representative of the population of patients with schizophrenia; rather, we selectively recruited a subset of patients with marked negative symptoms that justified a supplementary diagnosis of deficit syndrome. This aspect of the study design was dictated by our desire to maximize power to detect any true differences between the schizophrenic patients and the comparison subjects by minimizing heterogeneity within the patient group. However, it means that our results may not generalize to the population of patients with schizophrenia. Furthermore, the lack of variability in the patient group with respect to negative symptoms may account for our failure to demonstrate any significant anatomical associations with negative symptom scores. Third, all the patients were taking antipsychotic drugs both at the time of the study and for many years pre-

viously and, as noted earlier, antipsychotic medication is likely to cause changes in brain structure that can confound interpretation of case-control differences. This issue can only be resolved with certainty by future studies of antipsychotic drug-naïve patients. Fourth, we have presented most of our results in the form of hypothesis tests rather than confidence intervals. The use of hypothesis testing to summarize large quantities of imaging data is an almost universal practice but must be considered critically. Whether a region shows up as “significant” or not on an inferential brain map will obviously depend on the size of test applied to the data. We have here used very stringent probability thresholds, for which we expected less than one false positive test per map, to focus attention on the most salient (least questionable) effects of schizophrenia as expressed in this patient group. However, this means that the probability of type II errors will be considerable, or, more plainly, there may well be other abnormalities in brain structure than those we have reported.

In summary, we have used contemporary image analysis tools to demonstrate significant deficits of both gray and white matter in a refined group of patients with marked negative symptoms of schizophrenia. The anatomical abnormalities are spatially distributed in a network of neocortical and limbic regions and interconnecting white matter tracts. These data are compatible with supraregional models for the pathology of schizophrenia.

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