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Voxel-based morphometry versus region of interest: a comparison of two methods for analyzing gray matter differences in schizophrenia

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Abstract

Many previous studies exploring cortical gray matter (GM) differences in schizophrenia have used "region of interest" (ROI) measurements to manually delineate GM volumes. Recently, some investigators have instead employed voxel-based morphometry (VBM), an automated whole-brain magnetic resonance image measurement technique. The purpose of the current study was to compare the above methods in calculating GM distributions in schizophrenia patients relative to matched controls. Using ROIs, Buchanan et al. (Buchanan, R.W., Francis, A., Arango, C., Miller, K., Lefkowitz, D.M., McMahon, R.P., Barta, P.E. and Pearlson, G.D., 2004. Morphometric assessment of the heteromodal association cortex in schizophrenia. Am J Psychiatry. 161 (2), 322–331.) found decreased dorsolateral prefrontal GM volume and altered symmetry of inferior parietal GM in schizophrenia patients. We hypothesized that VBM analyses of the same data would complement the ROI findings. As predicted, VBM analyses replicated results of less left inferior and right superior frontal cortical GM in schizophrenia. Additionally, VBM uncovered a significantly lower concentration of GM in the middle and superior temporal gyri, sought but not detected using ROIs, but did not replicate the parietal changes. The principal explanation for these differences may be the methodological differences between voxel-averaged, landmark-based ROI analyses and the single, voxel-by-voxel whole brain VBM measurements. Although VBM is rapid and fully automated, it is not a replacement for manual ROI-based analyses. Both methods provide different types of information and should thus be used in tandem.
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Keywords: VBM; ROI; Schizophrenia; Gray matter; Heteromodal association cortex; MRI

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1. Introduction

The heteromodal association cortex (HASC) is comprised mainly of the dorsolateral prefrontal, superior temporal and inferior parietal cortices, and is hypothesized to be selectively involved in the neuropathology of schizophrenia (Baumann and Bogerts, 1999; Falkai et al., 2001; Keshavan et al., 2003; Mesulam, 2000; Pearlson et al., 1996; Schlaepfer et al., 1994). Magnetic resonance imaging (MRI) has been useful in revealing subtle structural brain abnormalities in patients with schizophrenia as compared to healthy controls. Imaging studies have associated schizophrenia with disproportionate gray matter (GM) volume reductions in HASC regions, prominent in the superior and medial temporal lobes, as well as the frontal and parietal cortices (Shenton et al., 2001).

The method used by many prior investigators to localize these GM disturbances in schizophrenia has been the measurement of manually delineated, anatomically defined regions of interest (ROI) within the brain. Frequently used, the ROI method has many strengths, namely anatomical validity. However, it also has limitations, including but not limited to the time-consuming nature of manual ROI drawings, both in delineating a priori-defined regions and in the rigorous training needed to ensure rater reliability, which does not easily allow for comparison of many brain regions or large subject groups (Kubicki et al., 2002). More recently, investigators have begun to employ voxel-based morphometry (VBM; Ashburner and Friston, 2000), a fully automated whole-brain measurement technique, to examine structural MR images of the brain. By surveying the whole brain, VBM provides a non-biased measure of highly localized regions that may not be investigated in hypothesis-based studies that employ more labor-intensive ROI measurement techniques. Defined by Ashburner and Friston (2000) as "a voxel-wise comparison of the local concentration of GM between two groups of subjects," VBM tests for residual tissue concentration differences that remain after all subjects MRI scans are spatially normalized into the same standardized stereotaxic space. GM is segmented out and then smoothed using convolution with a Gaussian kernel. Due to the nature of the normalization procedure, VBM analyses are less sensitive to shape

differences and thus may enjoy high reliability potentially at the expense of validity. They must therefore be employed with the caveat that errors in normalization may confound results. It is also important to distinguish between the relative within-voxel concentrations of gray matter (i.e. differences in the proportion of GM contained within a given voxel) as calculated by VBM and the absolute volumes revealed in ROI analyses. VBM results can be modulated to account for the variable shape changes in nonlinear normalization (Good et al., 2001), and thus preserve the volume of the particular tissue within a voxel. Modulated analyses test for regional differences in volumes of GM where standard unmodulated analyses test for regional differences in concentration of GM (Ashburner and Friston, 2000). We chose to perform the latter type of analyses, as both types of VBM analyses output relative measures of GM as opposed to the absolute volumes obtained in an ROI analysis.

A series of studies have now used VBM as a method to search broadly for brain regions showing variable amounts of GM in schizophrenics relative to healthy controls (Ananth et al., 2002; Gaser et al., 1999; Hulshoff Pol et al., 2002; Kubicki et al., 2002; Moorhead et al., 2004; Sigmundsson et al., 2001; Sowell et al., 2000; Suzuki et al., 2002; Wilke et al., 2001; Wright et al., 1999). These studies generally confirm and extend ROI studies (Gaser et al., 1999; Job et al., 2002) by increasing the anatomical range of volumetric comparisons. Such studies have shown that, relative to controls, schizophrenia samples exhibit decreased GM concentrations in the insula (Hulshoff Pol et al., 2002; Kubicki et al., 2002; Sigmundsson et al., 2001; Wright et al., 1999), thalamus (Ananth et al., 2002; Gaser et al., 1999; Hulshoff Pol et al., 2002; Sowell et al., 2000), cingulate gyrus (Hulshoff Pol et al., 2002; Kubicki et al., 2002; Sigmundsson et al., 2001; Sowell et al., 2000), all frontal gyri (Gaser et al., 1999; Moorhead et al., 2004; Suzuki et al., 2002; Wilke et al., 2001), superior temporal gyrus (Gaser et al., 1999; Hulshoff Pol et al., 2002; Kubicki et al., 2002; Sigmundsson et al., 2001; Suzuki et al., 2002; Wilke et al., 2001), and medial temporal lobe (Hulshoff Pol et al., 2002; Sigmundsson et al., 2001; Suzuki et al., 2002; Wilke et al., 2001). Some VBM studies have also demonstrated greater concentrations of GM among

schizophrenia samples in the basal ganglia (Gaser et al., 1999; Kubicki et al., 2002; Wilke et al., 2001) and cerebellum (Kubicki et al., 2002; Suzuki et al., 2002).

The current study had two main objectives. The primary aim was to use VBM to perform specific, age-matched contrasts within the main group comparisons. We hypothesized that these would reveal underlying differences based on sex and diagnosis. The second aim of this study was to evaluate VBM as a method of calculating GM disturbances in patients with schizophrenia as compared to the ROI method. We sought to explicitly assess the two methods by using VBM to analyze a data set previously analyzed using ROIs in HASC regions. Using the ROI method, Buchanan et al. (2004) found that schizophrenia patients exhibit reduced GM volume in the superior prefrontal and left supramarginal cortices, greater GM volume in the right supramarginal gyrus, and reversed asymmetry in the inferior parietal cortex relative to healthy controls. We hypothesized that a VBM analysis of this same data set would complement and extend the ROI findings.

2. Methods

2.1. Subjects

We used VBM to analyze MRI scans collected by Buchanan et al. (2004), and previously evaluated by ROI analyses. His report of these analyses by provides a detailed description of the methods used, as well as demographic information (included here in Table 1). Briefly, 41 patients (31 male, 10 female) with DSM-III-R/DSM-IV schizophrenia or schizo-affective disorder from an outpatient research clinic were selected for entry into the study. Patients were included based on a diagnostic approach that utilized all available information from the Structured Clinical Interview for DSM-III-R/DSM-IV (SCID; First et al., 1997; Spitzer et al., 1989), direct assessment, family informants, and past medical records. The 34 (17 male, 17 female) normal controls were recruited from a pool of community volunteers, and were also evaluated with the same structured interview as the patient group. Potential patients and control subjects were excluded if they had a history of organic brain

Table 1
Demographic data of participants included in the VBM study

	Normal controls (N=34)	Patients with schizophrenia (N=41)
Age at MRI (years; Mean±S.D.)	34.7±7.2	39.0±5.6
Gender (Male/Female)	17/17	32/12
Race (White/Black)	23/11	33/11
Age of onset (years; Mean±S.D.)	N/A	21.6±6.2*
Duration of illness (years; Mean±S.D.)	N/A	17.3±7.6*
Total brain volume (cc; Mean±S.D.)	1286.1±20.5	1273.2±19.0
Total cranial volume (cc; Mean±S.D.)	1403.0±23.2	1413.4±22.7

* N=38.

disorder, mental retardation, head injury with loss of consciousness for greater than 30 min, or a diagnosis of substance abuse of dependence within the last 12 months. Additionally, control subjects were excluded if they had a history of DSM-III-R/DSM-IV Axis I or Axis II disorder based on the SCID. Three male patients included in the ROI analysis were excluded from the VBM analysis, as the data were inadvertently deleted in the time between the ROI and VBM analyses. Forty-one of the original patient group and 33 controls were right-handed. All subjects performed written informed consent prior to study entry.

ROI analyses were performed by Buchanan et al. (2004), and the specifics of both region delineation and interrater reliability are detailed in their report of these analyses. Briefly, anatomical sulcal landmarks were used to delineate the prefrontal, superior temporal and inferior parietal regions, and interrater reliability measures, based on the independent measurement of each region in five brains, ranged from 0.86 to 0.99.

2.2. MRI image acquisition

The magnetic resonance images were obtained on a 1.5-T Signa GE scanner (GE Medical Systems, Milwaukee). The whole brain was evaluated in the coronal plane using a "spoiled" GRASS three-dimensional imaging sequence, with the following imaging parameters: 35 ms TR, 5 ms TE, 45° flip angle, 1

excitation, 1.5 mm slice thickness, 24 cm field of view, and a matrix size of 256×256.

2.3. Voxel-based morphometry

VBM analyses employed the computer program SPM2 (Statistical Parametric Mapping, developed by the Wellcome Institute, London, UK) running in MATLAB version 6.5 (The MathWorks, Natick, MA, USA). Images were analyzed using the standard VBM methods described in detail by Ashburner and Friston (2000), which involved spatially normalizing all of the images to the same stereotaxic space, extracting GM from the normalized images, smoothing, and performing statistical analyses to localize group differences. Processing components specific to this study will be emphasized here.

Individual coronal MRI images were automatically stripped of all non-brain tissue by Buchanan et al. (2004) using Brain Image (Reiss et al., 1995), and registered to a stripped template image by minimizing the residual sum of squared differences between them (Ashburner and Friston, 2000). The template was created by using the Brain Extraction Tool (Smith, 2002) to remove non-brain tissue from the standard T1 template in Montreal Neurological Institute (MNI) standard space included in the SPM2 software package. Normalized images were interpolated to voxel dimensions of 1.5×1.5×1.5 mm and then segmented into GM, white matter (WM) and cerebrospinal fluid (CSF) using a modified mixture model cluster analysis technique, with a correction for image intensity nonuniformity (Ashburner and Friston, 1997). Smoothing of GM images was performed by convolving with a 12-mm isotropic Gaussian kernel in order to compensate for the inexact nature of normalization, and to make the subsequent voxel-by-voxel analysis more comparable to the ROI approach. Each voxel in a smoothed image contains the averaged partial volume of GM from around and within the selected voxel (Ashburner and Friston, 2000), which also has the effect of rendering the data more normally distributed in accord with the central limit theorem, increasing the validity of later parametric statistical tests. Therefore, each voxel in the images included in the VBM analysis contains a concentration of GM volume, a value ranging from 0 (no GM) to 1 (all GM).

2.4. Statistical analyses

The processed images were analyzed using SPM2, which employs the framework of the general linear model (GLM). The two central one-sided group comparisons (*patients>controls* and *controls>patients*) were performed using a two-sample *t*-test analysis. Groups were not corrected for age or sex in the main comparisons, as we were interested in replicating the main effects ROI analyses performed by Buchanan et al. (2004). We also analyzed smaller group comparisons, organized by sex and diagnosis as follows: female controls vs. female patients, male controls vs. male patients, male patients vs. female patients, and healthy males vs. healthy females. As age and sex are known to influence brain morphology (Coffey et al., 1998; Schlaepfer et al., 1995), a simple regression analysis was performed to determine if the significant age effects in superior prefrontal and total GM volume found by Buchanan et al. (2004) emerged using VBM. Furthermore, the smaller groups were age-corrected. The resulting set of voxels from each contrast represents a statistical parametric map of the *t*-statistic (SPM-*t*).

SPM-*t* maps, comprised of results of statistical tests on each voxel, were all thresholded for the main group comparison of *all controls>all patients* at $p<0.005$ and corrected for multiple comparisons using the false discovery rate (FDR; Genovese et al., 2002). All contrasts were first corrected for multiple comparisons at $p<0.005$, and in the case of comparisons found to have no significant voxels, thresholds were lowered until trends emerged. The contrast of *all patients>all controls* required an uncorrected threshold of $p>0.001$ to elicit significant voxel-based differences. Main sex differences were also explored in the contrasts of *all females>all males* ($p<0.005$, corrected), and *all males>all females* ($p<0.01$, uncorrected). Four contrasts *(all patients>all controls, male patients>female patients, male patients>male controls,* and *female controls>female patients)* only elicited significant voxels when uncorrected.

For visualization of group differences, the SPM coordinates and significant voxels were superimposed onto SPM2's spatially normalized template brain. The coordinates of the most significantly different voxels were transformed from the MNI coordinate system to the coordinates of the standard space of Talairach and

Tournoux (1988) using a MATLAB conversion program written by Matthew Brett (MRC Cognition and Brain Sciences Unit, Cambridge, England). Once converted, the Talairach coordinates were entered into the Talairach Daemon (Lancaster et al., 2000) for result localization.

3. Results

3.1. Main group contrasts

In a voxel-by-voxel contrast, after correcting for multiple comparisons, several regions showed significantly ($p<0.005$, corrected) lower within-voxel concentrations of GM in schizophrenia patients relative to healthy control subjects. Table 2 details relative volumes and Talairach coordinates of the significant areas briefly listed here: the bilateral frontal, superior temporal, cingulate, insula, and thalamus, and the right subcallosal and postcentral gyri. No region showed significant differences in patients versus controls when corrected for multiple comparisons, but when the threshold was lowered to an uncorrected $p<0.001$, the right rectal, left inferior temporal and parahippocampal gyri demonstrated a trend towards higher GM concentration (Table 2) (Fig. 1).

To replicate the GM volume effects by sex examined by Buchanan et al. (2004), we contrasted *all males>all females*. When correcting for multiple comparisons at any p-value, no brain regions showed any significant differences in males over females. However, with an uncorrected $p<0.01$, the left cuneus, inferior occipital, lingual and anterior cingulate gyri demonstrated a trend towards a significant sex effect of males greater than females. The reverse contrast of *all females>all males* was much more significant, with the bilateral superior, medial and precentral frontal gyri, and the right cuneus, inferior parietal and middle occipital gyri demonstrating significantly greater GM concentrations ($p<0.005$, corrected) in females relative to males (Table 3).

3.2. Group-specific contrasts

In the interest of performing specific, age-matched comparisons based on sex and diagnosis, we divided the main groups into the following contrasts ($n=$in each group; $\mu_{age}=$average age): healthy males vs. healthy females ($n=16$, $\mu_{age}=43$), male patients vs. female patients ($n=10$, $\mu_{age}=48$), male controls vs. male patients ($n=15$, $\mu_{age}=44$), and female controls vs. female patients ($n=8$, $\mu_{age}=47$). To parse out the diagnosis-based differences seen in the main sex

Table 2
Summary of areas detected in the main effects analyses

Brain region	Brodmann's area	Volume (cc)		Max T (x,y,z)	
		L	R	L	R
Areas with lower GM concentration in patients relative to controls: ($p<0.005$, corr.)					
Superior, middle frontal gyri	6, 8, 9, 10, 46	7.0	9.2	6.0 ($-13,56,-8$)	5.6 (22,59,5)
Inferior, precentral frontal gyri	6, 9, 44, 45, 47	5.2	4.6	5.5 ($-56,23,3$)	5.2 (45,16,-3)
Subcallosal gyrus	25	0.1	–	4.1 ($-3,8,-11$)	N.S.
Postcentral gyrus	43	0.1	–	4.2 ($-67,-9,14$)	N.S.
Insula	13	0.5	1.4	4.8 ($-49,10,-2$)	5.0 (46,10,-2)
Superior temporal gyrus	13, 22, 38, 42	2.5	1	5.1 ($-46,13,-7$)	5.1 (45,10,-4)
Cingulate gyrus	10, 24, 25, 32, 33	4.7	3.3	5.4 ($-1,4,-4$)	5.2 (4,23,24)
Thalamus (MDN, pulvinar)		1.1	0.8	5.5 ($-3,-17,4$)	5.4 (4,-19,6)
Areas with higher GM concentration in patients relative to controls: ($p<0.001$, uncorr.)					
Rectal gyrus	11	–	0.1	N.S.	3.5 (10,31,-19)
Inferior temporal gyrus	20	0.4	–	3.6 ($-56,-26,-19$)	N.S.
Parahippocampal gyrus	36	0.3	–	3.5 ($-36,-20,-20$)	N.S.

A selection of areas detected by the main diagnosis analyses *(controls>patients* and *patients>controls)* along with their Talairach coordinates, volume and the maximum *t*-value and its coordinate. For all tables, each volume listed is the size of the cluster of contiguous voxels above the given threshold in each area.
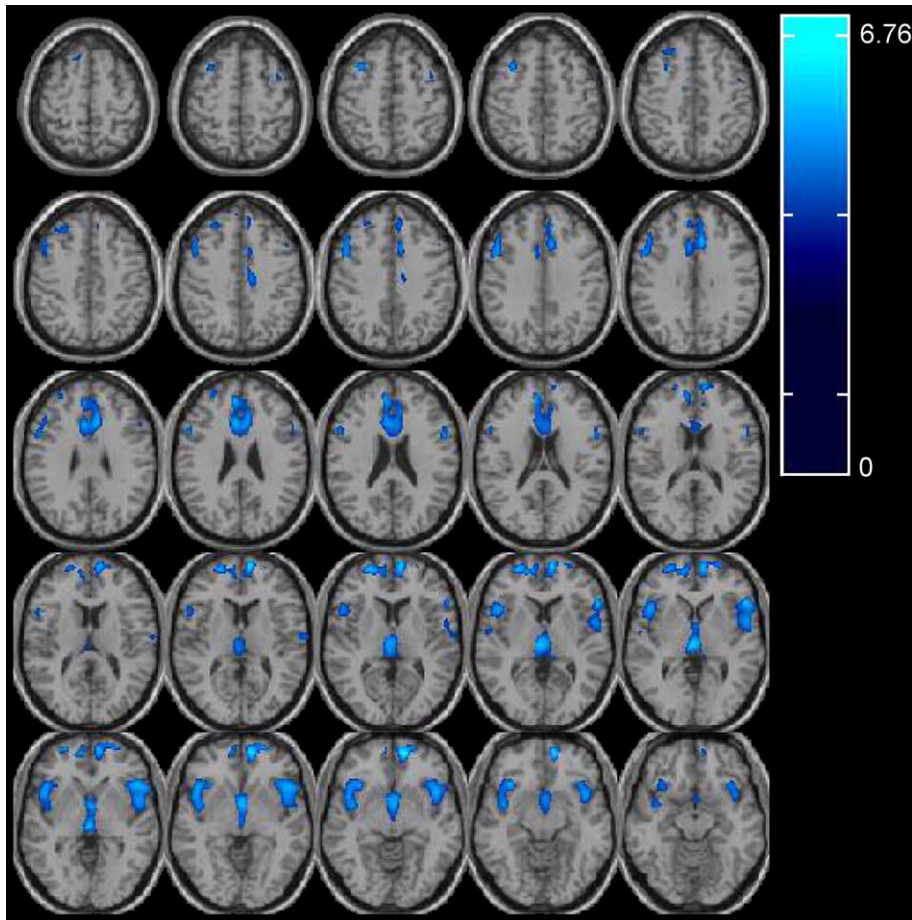
Fig. 1. Main effect group map of volumes with lower concentrations of GM (blue, corrected $p<0.005$) in schizophrenia patients relative to healthy controls.

comparison, we compared the contrasts of male controls vs. female controls with the same contrast composed of patients only. Where healthy females only demonstrated slightly higher concentrations of GM in the left superior frontal gyrus as compared to healthy male subjects ($p<0.005$, corrected), female schizophrenia patients showed many more areas with larger GM concentrations than male patients ($p<0.005$, corrected): bilateral superior frontal, medial frontal, precentral, paracentral, postcentral, superior parietal, and inferior parietal gyri, bilateral precuneus and right cuneus. Males showed the opposite effect, with the healthy controls demonstrating a trend towards higher concentrations in the left cuneus, insula, middle occipital, inferior occipital,

lingual, and middle temporal gyri as compared to healthy females ($p<0.01$, uncorrected). When contrasted with matched females, male patients only had a trend towards greater GM concentrations in the right medial frontal and anterior cingulate gyri ($p<0.01$, uncorrected). These differences are detailed in Table 3.

Other smaller contrasts performed sought differences correlated with diagnosis within sex: male patients vs. male controls, and female patients vs. female controls. The decreases in the concentrations of GM of male patients relative to male controls became visible at a corrected $p<0.001$, and encompassed the right superior and medial frontal, insula and superior temporal gyri, and left cingulate gyrus.

Table 3
Within diagnosis sex differences table

	Vol (cc)		Max T (x,y,z)		Vol (cc)		Max T (x,y,z)		Vol (cc)		Max T (x,y,z)	
	L	R	L	R	L	R	L	R	L	R	L	R
Females more GM	ALL (p<0.005)				CONTROLS (p<0.005)				PATIENTS (p<0.005)			
Superior frontal gyrus	0.7	0.1	5.7 (−12,66,6)	5.4 (9,15,61)	0.2	–	5.4 (−10,64,6)	N.S.	0.2	0.5	7.0 (−18,−14,66)	6.4 (10,13,60)
Medial frontal gyrus	0.7	0.3	6.0 (−6,53,37)	5.8 (6,63,12)					0.5	0.7	5.9 (−3,−18,55)	6.1 (3,−16,55)
Precentral gyrus	0.4	0.2	5.4 (−42,−10,38)	5.4 (43,−11,38)					2.3	4.6	11.3 (−18,−17,64)	7.9 (34,−23,58)
Paracentral lobule	0.1	–	5.8 (−16,−31,50)	N.S.					1.1	1	6.2 (−15,−35,57)	6.1 (12,−32,55)
Postcentral gyrus									2.7	3	7.8 (−15,−40,66)	6.7 (61,−22,36)
Superior parietal lobule									0.2	0.6	6.1 (−36,−62,45)	7.6 (31,−63,47)
Inferior parietal lobule	–	0.3	N.S.	5.6 (48,−46,51)					1.1	0.1	6.7 (−39,−59,46)	5.3 (33,−37,56)
Precuneus									0.8	1	6.3 (−4,−47,58)	5.8 (1,−48,58)
Cuneus	–	0.6	N.S.	6.5 (13,−85,19)					–	0.4	N.S.	6.0 (4,−74,29)
Middle occipital gyrus	–	0.2	N.S.	6.4 (12,−87,17)								
Males more GM	ALL (p<0.01)				CONTROLS (p<0.01)				PATIENTS (p<0.01)			
Medial frontal gyrus									–	0.1	N.S.	3.3 (15,47,3)
Cuneus	0.8	–	2.9 (−6,−96,5)	N.S.	1.4	–	4.0 (−10,−96,0)	N.S.				
Middle occipital gyrus					0.1	–	3.8 (−37,−82,−7)	N.S.				
Inferior occipital gyrus	0.1	–	2.5 (−42,−91,−8)	N.S.	2.3	–	4.8 (−40,−86,−8)	N.S.				
Insula					0.3	–	3.6 (−36,−25,21)	N.S.				
Lingual gyrus	1.2	–	2.8 (−4,−92,0)	N.S.	3.5	–	4.3 (−12,−92,−3)	N.S.				
Middle temporal gyrus					0.1	–	3.2 (−59,−38,−2)	N.S.				
Anterior cingulate	0.1	–	2.6 (−15,37,4)	N.S.					–	0.3	N.S.	4.3 (13,39,−1)

Comparison of areas detected in the main sex contrasts for *all males>all females* and *all females>all males* with same contrasts grouped by diagnosis, along with their Talairach coordinates, volumes and the maximum *t*-values with their coordinates. Many of the areas shown as larger in all females over all males are also present in the same contrast consisting of patients, while many of the areas bigger in all males than all females are present in the control contrast. All of the *p*-values listed for females are corrected for multiple comparisons (FDR), while none of the males are.

There were no brain regions that exhibited differences in male patients relative to male controls at any corrected p value, nor any higher GM concentrations in female schizophrenia patients relative to female controls. Female patients, however, demonstrated a significantly larger concentration of GM relative to female controls at a corrected $p<0.001$ in the following areas: bilateral superior, middle and inferior

Table 4
Within sex diagnosis differences table

Areas with higher concentrations of GM in:	Vol (cc)		Max T (x,y,z)		Vol (cc)		Max T (x,y,z)	
	L	R	L	R	L	R	L	R
	MALES: C>SZ ($p<0.001$, corrected)				FEMALES: SZ>C ($p<0.001$, corrected)			
Superior frontal gyrus	–	0.1	N.S.	4.4 (9,28,43)	1.0	1.6	4.0 (−16,41,−20)	4.9 (16,34,−21)
Medial frontal gyrus	–	0.1	N.S.	4.8 (9,26,44)				
Orbital gyrus					0.1	0.6	3.4 (−18,38,−23)	4.7 (16,35,−23)
Precentral gyrus					0.1	0.8	3.3 (−27,−20,67)	4.8 (13,−17,66)
Postcentral gyrus					1.4	0.8	4.3 (−24,−32,60)	4.6 (31,−35,56)
Inferior parietal lobule					0.1	0.4	3.4 (−33,−48,54)	4.6 (62,−25,33)
Precuneus					1.6	2.8	4.2 (−10,−71,38)	5.1 (9,−78,38)
Cuneus					0.4	1.8	4.1 (−21,−92,−2)	5.2 (10,−84,32)
Lingual gyrus					3.2	1.1	4.8 (−25,−91,−6)	3.9 (3,−85,−6)
Insula	–	0.1	N.S.	4.4 (40,1,−9)				
Superior temporal gyrus	–	0.1	N.S.	4.5 (39,2,−11)				
Middle temporal gyrus					0.8	0.1	4.0 (−36,−82,−9)	3.5 (36,−72,−10)
Inferior temporal gyrus					2.4	–	5.1 (−31,−88,−6)	N.S.
Cingulate gyrus	0.1	–	4.2 (−9,16,35)	4.0 (4,20,28)	–	0.4	N.S.	3.6 (4,−56,7)

Comparison of the areas detected in the *patients>controls* and *controls>patients* contrasts for both sexes, with their Talairach coordinates, volumes and the maximum *t*-values and their coordinates. Only the male controls>male patients and female patients>female controls contrasts demonstrated significantly different volumes at the thresholds listed.

frontal, orbital, precentral, postcentral, paracentral, inferior parietal, precuneus, cuneus, lingual, fusiform and middle temporal gyri, the left inferior temporal gyrus and the right posterior cingulate gyrus. Relative volumes of GM concentration differences and Talairach coordinates are shown in Table 4.

4. Discussion

4.1. Within-group VBM contrasts

The main effects analyses demonstrated significantly lower concentrations of GM in frontal, temporal and sub-cortical GM regions in schizophrenia patients as compared to healthy controls, as well as higher concentrations of GM in the right rectal, left inferior temporal and parahippocampal gyri. The all female grouping exhibited significantly higher concentrations of GM than males in bilateral frontal, parietal, occipital and cingulate gyri, while the all male grouping demonstrated a trend towards higher concentrations in certain occipital volumes (see Table 3). These data support previous findings from our group and others that although GM proportions are similar globally between the sexes, females show proportionately higher GM concentrations in portions of the frontal and the right parietal lobe than males (Frederikse et al., 2000; Nopoulos et al., 2000; Schlaepfer et al., 1995).

Given the documented disruptions in GM volume in schizophrenia, the grouping of patients with controls to measure "sex effects" is potentially risky. As almost half of the female group consisted of patients with diagnosed schizophrenia, we hypothesized that some of the differences seen in the *all females>all males* contrast might be confounded by diagnosis effects. To test this, we thresholded the three contrasts of *all females>all males, control females>control males,* and *female patients>male patients* at a corrected $p<0.005$ in order to directly compare the findings. As predicted, the *control female>control male* contrast elicited differences in only the left superior frontal gyrus. Conversely, the patient contrast showed significantly larger concentrations of GM in more diffuse frontal and parietal areas in females than males (see Table 3). Therefore, it seems that the greater concentrations seen in female patients' cortical GM as compared to the lack of differences in the healthy controls influenced the main *all females>all males* sex contrast.

The *all male>all female* contrast failed to reveal any significant local differences in GM at $p<0.005$. As healthy males are reported to have larger total intracranial volumes than females, equally distributed among all four lobes (Nopoulos et al., 2000), we hypothesized that alterations in the GM distributions in the patient population might have confounded the data, and performed the same comparison of contrasts as we did in the *female>male* group of contrasts. However, since none of the contrasts were significant at the threshold set for other contrasts ($p<0.005$), the areas identified in male comparisons are based on trends. The *male>female* contrasts reveal an opposite pattern than the *female>male* ones, in that the control contrast identifies many more areas with significantly greater proportions of GM in healthy males than healthy females than the schizophrenia contrast. In healthy controls, we found, in support of Buchanan et al. (2004), that males generally had higher regional GM concentrations, mostly in the left hemisphere. In contrast, the areas higher in male patients than female patients were on the right. Furthermore, we acknowledge that the inherent differences in brain size within this sample may affect the amount of warping needed to register each image to the standard T1 template. However, the *x*, *y*, and *z* scaling parameter directions (i.e. stretching or shrinking in each direction) are not all in agreement, and thus it is unlikely that they could account for the differences seen in the VBM contrasts.

We also performed within-sex diagnosis-based analyses, and compared male and female patients with male and female controls in Table 4. While the healthy males demonstrated significantly greater concentrations of GM in right frontal and temporal areas than male patients, no areas were higher in male patients than controls. Again, the opposite pattern emerges in females, with the *female control>patient* contrast revealing no differences and female patients exhibiting significantly greater bilateral frontal, parietal, visual and temporal proportions of GM than healthy controls (Table 4).

Comparing these patterns, the data reveal that the diagnosis of schizophrenia is strongly correlated with greater frontal and parietal GM concentrations in females, but lesser occipital and temporal GM in males. These sex differences in schizophrenia are likely due to an interaction of the disease process, genetic and hormonal differences, variations in the maturation and morphology of the brain, and sex-specific behavioral patterns (Hafner, 2003; Pearlson, 2000). Although there are no definitive answers about sex differences in schizophrenia, women have been found to display more positive symptoms, have a more benign illness and a later stage of onset than men, who also present with more negative symptoms (Maric et al., 2003). Further analysis of these data may provide additional evidence of neural sex differences in schizophrenia that possibly contribute to the differential clinical disease expression in men and women (Frederikse et al., 2000; Goldstein et al., 2002).

As for age effects, it must be noted that the average age of the patients (overall average 46.63 ± 6.07 years) was significantly ($p<0.01$) higher than the average age of the control subjects (overall average 42.53 ± 6.89 years). Buchanan et al. (2004) reported significant age effects in superior prefrontal and total GM volume, supporting previous studies (Gur et al., 2002) findings of increasing GM loss in the frontal, temporal and basal ganglia regions with age. Past use of VBM to examine age effects has also found significant GM loss in bilateral parietal, insula, and anterior cingulate gyri with increased age (Good et al., 2001). We performed a post hoc VBM analysis on this data set to replicate Buchanan's findings, and found

that in the prefrontal and cingulate cortices, GM concentration is negatively correlated with age. In addition, we also repeated the main patient vs. controls contrasts while controlling for age, and found that the results were not significantly affected. Therefore, although the patient group was significantly older than the controls, the natural effects of aging did not significantly confound the differences in GM attributed to the presence of schizophrenia in this study.

4.2. VBM vs. ROI

Many previous studies have examined the disruption of GM volume in patients diagnosed with schizophrenia, using both traditional ROI-based as well as newer VBM methods. The results obtained in this analysis replicate those from several other VBM and ROI studies, namely Hulshoff Pol et al. (2002) and Kubicki et al. (2002). The main replication is less GM in schizophrenics relative to healthy controls in the left superior frontal gyrus (Gaser et al., 1999), left and right inferior frontal gyrus (Buchanan et al., 1993; Suzuki et al., 2002), left and right superior temporal gyrus (Barta et al., 1990; Kubicki et al., 2002; Shenton et al., 1992), left insula (Crespo-Facorro et al., 2000; Hulshoff Pol et al., 2002), right insula (Kubicki et al., 2002), bilateral anterior cingulate gyrus (Harris et al., 1991; Kubicki et al., 2002), and bilateral thalamus (Hulshoff Pol et al., 2002). There was also a trend (VBM $p<0.001$, uncorrected) of greater GM concentrations in schizophrenia in the right orbital gyrus, which replicates the ROI finding of Buchanan et al. (2004). We acknowledge that the interface between bone, brain and air in the orbitofrontal area can suffer from susceptibility artifact, however we manually inspected the images and they appear normal.

The second aim of this study was to explicitly compare the results of this VBM analysis to the results of the ROI analysis on the same data set analyzed by Buchanan et al. (2004), which found patients to have reduced GM volume in the superior prefrontal, left inferior parietal and left supramarginal (trend, $p<0.07$) cortices, and larger right supramarginal GM volume. We replicated the ROI finding of significantly smaller bilateral superior prefrontal volume, but no significant differences in the parietal

lobe emerged at a corrected $p<0.005$. The larger volume of GM in the right supramarginal gyrus seen in the ROI analysis was also not replicated. However, both the ROI and VBM analyses revealed significantly smaller amounts of GM in male patients as compared to controls, namely in the prefrontal cortex. As mentioned before, although the values for the other prefrontal regions are not listed in the ROI results, using VBM we also found a trend towards lower GM concentrations in the right frontal and temporal gyri, as well as the left cingulate gyrus in male patients versus their matched controls (Table 4). The finding of less frontal and temporal GM in part supports previous findings (Pearlson et al., 1996) of reduced HASC volumes in schizophrenia. It must be noted that because data from three of the male schizophrenia patients included in the ROI study were corrupted and thus unusable upon arrival at our lab that the sample sizes vary slightly.

4.3. Conclusion

The primary cause for the discrepancy between ROI and VBM results likely stems from the methodological differences between voxel-averaged, landmark-based ROI analyses and the single, voxel-by-voxel whole brain VBM measurements. Although both methods are currently being employed by researchers, each has unique strengths and limitations. Manually delineated regions of interest are subject to inaccuracies, as local individual neuroanatomy is highly variable, especially in a diseased population, and thus more subject to error when regions are unusually small, or depart from usual conformation patterns, and are thus potentially difficult to define. The hypothesis-based nature of the ROI method makes it possible to only search for differences in cortical volume in pre-specified areas like the heteromodal association cortex investigated in the Buchanan study. However, ROI analyses enjoy substantial anatomic validity and output absolute accountings of the number of voxels in the regions being investigated, as compared to the relative changes in the concentration of GM within each voxel provided by VBM results. Although not as discrete as ROIs, the VBM method gives equal weight to every voxel included in the 3D sampling array, and thus provides a broad assortment of

regional comparisons. Nevertheless, the normalization procedures employed, although highly reliable, render the process less sensitive to group differences in shape or gray-white matter differentiation, and are prone to errors caused by misregistration of anatomical structures (Ashburner and Friston, 2001; Bookstein, 2001). Here, the assumption is made that schizophrenia and control brains can be equally forced into standard stereotactic space; this is an unproven assumption that may add error. It is important to note that the removal of global size differences in the normalization process is a caveat in all VBM studies, especially in those examining populations like schizophrenia where brain atrophy is documented. The method has been updated and optimized recently (Good et al., 2002) to reduce errors due to systematic differences in head shape, variations in segmentation, inconsistent brain stripping, and errors introduced by spatial normalization. However, as the validity of VBM has been questioned but not yet documented (Kubicki et al., 2002), different hypotheses may require different VBM parameters, and results must be viewed with caution.

Therefore, VBM must be employed with the caveat that errors in normalization may confound the later analyses. The combination of slight errors both in the ROI and VBM analyses may account for some of the different results obtained, especially in regions susceptible to schizophrenia-related GM loss. These results support previous suggestions (Kubicki et al., 2002) that although VBM is rapid and fully automated, it is not a replacement for traditional ROI-based analyses. Both methods provide different types of information and should thus be used in tandem (Kubicki et al., 2002), with VBM being used to quickly generate hypotheses by identifying areas that can subsequently be more thoroughly investigated using ROI-based approaches.

Acknowledgements

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