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Schizophrenia: neural architecture, brain regional differences, and changes with age

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Abstract

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Schizophrenia is a heterogeneous and life-altering disorder. Although a multitude of research has been published on schizophrenia since its first iteration as *dementia praecox*, there is still much to be learned about this disease. Neuroscientific research has revealed important findings on brain changes; some of these have become consistent with the disease, such as the decrease in whole-brain gray matter and the enlargement of the ventricles. However, other findings remain controversial, including the posited increase in neuron density. New techniques are being implemented every day to study these changes, including the use of diffusion tensor imaging. A novel application of this method in the gray matter in the current study reveals increased diffusivity in schizophrenia, potentially implicating decreased tissue integrity and a loss of neuropil. Relationships with asymmetry are also explored in conjunction with altered neurodevelopment. An examination of the microstructure literature using meta-analysis suggests that overall there is an increase in density in schizophrenia. This provides support for a neurodevelopmental origin of schizophrenia, and has implications for the reduced neuropil hypothesis; additionally, a finding of decreased inhibitory neurons in schizophrenia provides support for the theory of dysfunctional inhibitory cortical circuits. A focus on the microstructure in the inferior parietal lobule, a neglected region in schizophrenia research, reveals a difference in structure in schizophrenia, as well as important relationships with age that support a progressive course to the disease. The

effect of clinical variables such as medication and length of illness were examined in each of these studies, and these relationships provide important insight into the progression of the disease, as well as the nature of the impairment in those who have schizophrenia. The various techniques used in this project combine to create a picture of profound and comprehensive alterations in brain structure at both the macro- and micro-scale.

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In the end, it comes down to this.

Collaborations

A short-term visiting student in Dr. Chance's laboratory gathered pilot data for the meta-analysis of Chapter Two prior to the start of my Masters research; however, this was superseded by my own data collection. The Statistical Consultancy Service was consulted for advice on how to proceed with the multi-level statistical model of Chapter Two; Dr. D. Lunn of the Consultancy Service helped me to create this complex model.

Introduction

Schizophrenia is a complex mental disorder that is characterized by changes in behaviour and cognition; volumetric and histological studies have confirmed that there are a multitude of brain changes that occur in schizophrenia that differentiate patients from control subjects. However, there is no signature pathognomic pattern of abnormalities that has been identified that is characteristic of all occurrences of the disease.

With the advent of more precise and less expensive magnetic resonance imaging technology, our understanding of what is going on at a macroscopic level in the schizophrenia brain has increased rapidly in the last several years. In 1976, a landmark computerized axial tomography (CT) study was the first imaging study to show that patients have larger ventricles than controls[1]. Since then, there has been a wealth of studies and meta-analyses looking at differences in structure size, both between schizophrenia patients and healthy controls, and longitudinally within schizophrenia patients. These studies have shown that between patients and controls, there are often larger ventricles in schizophrenia, as well as decreases in both whole brain and gray matter volume[2-9]. Regional volumes have also been shown to be altered in schizophrenia, including a decrease in gray matter of the fusiform gyrus[10], insula and parahippocampus[11], hippocampus[12], thalamus and caudate nucleus[13], as well as gyri in the frontal and temporal cortices[6,13,14]. This reduction is not always consistent across all regions, however, as nonsignificant changes in volume have been reported in regions including the caudate nucleus[5] and the planum temporale[15].

DTI

Recently, studies have utilized the technique of diffusion tensor imaging (DTI) for investigating brain differences in schizophrenia: it allows for an *in vivo* investigation of the diffusion, voxel-voxel, in any given area in the brain. DTI is a magnetic resonance imaging method that uses the diffusion properties of water to show differences between types of brain tissue. Diffusion can be either isotropic (in all directions equally) or anisotropic (preferentially in one direction); in white matter tracts, diffusion is highly anisotropic, as the tracts constrain the diffusion to certain pathways. Cerebrospinal fluid is highly isotropic, as there is little underlying structure to constrain the diffusion. Most of the DTI studies that have been performed in schizophrenia have explored the white matter; this provides important information about tracts and connectivity in schizophrenia, and how they are related to functional networks and deficits. Values such as fractional anisotropy and mean diffusivity can be extracted from DTI investigations, and can be used to predict the organization of the underlying cortex. Fractional anisotropy measures the degree of anisotropy in each voxel, averaged over a given area; mean diffusivity reports the average amount of diffusion in a given area. DTI provides an elegant way to use noninvasive imaging techniques *in vivo* to focus on regions of interest, and therefore to gain more detailed information than perhaps MRI or other imaging techniques would allow[16]. In general, decreases in fractional anisotropy (FA) have been reported in schizophrenia[17], particularly in the left frontal[18-24] and temporal lobes[24-29]. However, auditory hallucinations[30-32] have been associated with increased anisotropy. To date, much of the DTI research has focused on the white matter; this highlights a need for these studies to be performed in the gray matter, and will be discussed further in chapter one.

MRI & Histology

As noted, MRI and DTI studies in schizophrenia have yielded several significant findings, including regional differences in gray matter volume, and changes in ventricular volume and white matter density[11,13,33]. Although these macroscale techniques provide needed information about overall brain and regional changes, histology studies are necessary to provide detailed examinations of the underlying microstructure in schizophrenia. Previous histological studies have traditionally focused on the prefrontal cortex and the temporal cortex. Processes such as reasoning and higher cognition, which are often altered in schizophrenia, are primarily attributed to the prefrontal cortex; the temporal lobe is investigated in schizophrenia due to its association with positive symptoms[34,35], including auditory hallucinations[36] and thought disorder[37]. Histology studies in these regions have often focused on neuron density[38-40] and alterations in laminar structure[15,41,42]. Differences in neuronal cell number or neuropil, as measured in histology studies, can support and justify results of volumetric differences seen via MRI. Previous histological studies in schizophrenia have found that there is increased neuron density in prefrontal cortex[43-45], as well as decreased density in the hippocampus[38], planum temporale[46], fusiform cortex[47], and parahippocampal gyrus[48]. However, these changes are not always consistent across regions, and in some cases contradictory findings have been reported, in both the frontal and temporal cortices[40,49-52].

Several candidate microstructural components have been put forth as substrates to account for the brain differences between schizophrenia patients and controls, including synapses, dendritic branches, and minicolumns. In 1990, Masliah et al[53] described a new method of measuring synaptic density by using immunohistochemistry

to measure the density of synaptophysin, a protein present in presynaptic terminals. Since then, several studies have used this protein in order to quantify synapses in different areas in schizophrenia: decreased synaptophysin has been found in the medial temporal lobe[54], frontal and visual cortices[55], and cingulate gyrus[56]. Several presynaptic markers, including synaptophysin[54], SNAP-25[57] (synaptosome-associated protein 25kDa), and synapsin[58] have been found to be decreased in the hippocampus in schizophrenia[59]. Decreased synaptic vesicle proteins are also found in the thalamus, cingulate gyrus, hippocampus, and frontal and parietal cortices[58]. However, these findings are not conclusive: other studies have found no change in synatophysin[60] or synaptophysin mRNA[61] in the prefrontal cortex of patients, and no change in synaptic vesicle proteins in the temporal cortex or cerebellum[56].

A decrease in dendritic spines has been found in both frontal and temporal association areas[62], as well as in the orbitofrontal cortex[63]. This decrease in spines seems to occur without a corresponding decrease in neuron number: it has been shown in both the prefrontal and occipital cortices that neuron number is not decreased in schizophrenia, and density of neurons in fact increases[43]; this has also been shown in the primary auditory cortex[64]. This is significant because the reduced neuropil hypothesis[65] posits that over time in schizophrenia, the amount of neuropil decreases, mediated by changes in dendrites, axons, and terminals. This results in an increase in neuronal density, relative to controls, without a concomitant increase in neuron number. Reduced neuropil may explain the contradiction between reduced brain volume over time in schizophrenia and the seemingly incompatible microanatomical findings of decreased spines with no change in neuron number: if it is the neuropil that is reducing in schizophrenia, then it would not be necessary for there to be a reduction in neuron number for there to be a reduction in brain volume. However, recently

Kreczmanski et al[66] found reduced neuron number in the caudate nucleus, the putamen, and the lateral nucleus of the amygdala, with no change in neuron density; similar findings have been shown in the visual cortex[67] and the pulvinar[68]. As the neuron density is unchanged, this indicates that, at least in these areas, there may be a decrease in neuron number along with a proportionate decrease in volume of neuropil, the space between cells. Smiley et al[15,49] reported decreased cortical width in the planum temporale in schizophrenia but no associated increase in neuron density. This does not support the reduced neuropil hypothesis: if the decrease in width was due to a decrease in neuropil, density should have increased. Instead, these results indicate that there may be a decrease in neuron number in this region in schizophrenia. The discrepancy between changes in neuron number and neuropil will be the topic of chapter two.

Although measures of dendritic spines and branches are useful, as they provide detailed information about the synaptic microstructure, other histological measurements can also provide information about the arrangement of this microstructure, and how it differs in schizophrenia. Minicolumns are structural units of microanatomy that form during development[69]; they are useful as a tool to investigate changes in the cortical structure. A minicolumn, which is the smallest level of modular organization, consists of cells that are oriented in a columnar fashion, with a cell-dense core surrounded by neuropil space. The radial unit hypothesis, as described by Rakic[70] describes how minicolumns form: cells originating from the ventricular zone migrate to the cortical plate via either a glial or axonal pathway, pass previously migrated cells from the same origin, and settle behind them, forming a column. These columns can be clearly seen in histological sections. Since minicolumnar structure is formed during development, alterations in minicolumns may present as the underlying structural feature behind regional and whole brain changes[71]. Previous studies that measured

minicolumnar structure found that minicolumn thinning correlated with increased age in controls in the planum temporale[41] and the middle temporal gyrus[72]. In the fusiform gyrus, minicolumns were found to be wider in patients than in controls; there was also thinning with age in controls in this region, and no association with age in patients[47]. This leads to the interpretation that patients have altered neuroplasticity over time, as indicated by changes in the microstructure; changes in neuropil may manifest in different ways. Minicolumns and altered neuroplasticity will be examined in the third chapter.

Changes with Age

The neurodevelopmental hypothesis, synthesized by Weinberger[73] in 1987, posits that there is a lesion in the brain that affects later neuroplastic events in life, most notably those caused by sexual maturation, which then leads to schizophrenia. There is strong continued support for this model throughout the literature[74,75]. The primary arguments come from findings that many of the changes that are seen in schizophrenia can be traced back to an alteration in development[76], such as changes in cytoarchitecture[51,71] and cerebral asymmetry[77,78]; brain changes in first-episode patients and those at high risk support this argument[79-81]. A primary tenet of the neurodevelopmental model is that there is stability of cognitive function later in the illness, after an initial decline[82,83]. Although the model has been changed and adapted with new research, it remains an important hypothesis in the field[74,75]. However, the case for progressive brain changes is also strong: several recent reviews and meta-analyses have provided evidence that there is progressive decline in brain volumes and increases in ventricular volume, alongside decreases in white matter integrity, which in some cases have been correlated with worse clinical outcome[2,4,84,85].

Recently, DeLisi[86] used modern imaging techniques to synthesize data to support the theory that schizophrenia might be a progressive disease. A meta-analysis of longitudinal imaging studies showed that there are progressive decreases in whole-brain volumes and whole-brain gray matter volume, as well as frontal, parietal, and temporal lobe volumes over time in schizophrenia[2]. Ongoing brain volume loss in the prefrontal cortex has been found during the transition from at-risk to first-episode schizophrenia[87]; progressive decreases in auditory cortex volume have been observed in first-episode patients[88], as well as increases in ventricular volume over time[4].

Progressive changes with age have been shown in the first 20 years of illness, with an altered trajectory of gray matter decrease in patients compared to controls[89]; this trajectory was found to be linear in patients, and curved in controls[90]. An altered trajectory of gray matter decrease has also been found in childhood-onset schizophrenia[91,92], as well as in first-episode psychosis[3,93], where decreases in volume were related to outcome after two years and cumulative antipsychotic medication. Studies of cognitive function in schizophrenia have reported that there is a decline later in life[94,95], in contradiction with previous findings[83]; decreased gray matter density has also been correlated with poor outcome and more severe symptoms later in the illness[13,89]. Changes with age have been noted in the frontal lobes, with progressive reduction in frontal lobe volume and an increase in cerebrospinal fluid in patients at a different rate than controls[95], as well as in the temporal lobes, with a significant decrease in white matter area in the superior temporal gyrus in older patients compared to older controls[96]. A decrease in cortical thickness has also been found with age in patients compared to controls[97].

A unifying hypothesis has emerged that rectifies these seemingly contradictory views: the progressive neurodevelopmental model suggests that onset of schizophrenia might be preceded by an alteration in neurodevelopment that is affected by maturational processes, and later interacts with neuroplastic processes in a way that is different from healthy aging[43,76,86,98]. Even though there is support for this hypothesis, there are not many studies that have investigated this in the microanatomy. Since the minicolumnar structure forms during development[71], changes between patients and controls on this measure could indicate the development of the different aging trajectory seen in schizophrenia; this would also be supported by progressive changes in other aspects of the microstructure, including neuronal organization.

Clinical variables: illness duration, age of onset, gender and medication

The effects of illness duration, age of onset, gender and brain morphology are intertwined throughout the literature. There have been findings of increased and more prominent brain changes in schizophrenia associated with a longer duration of illness, including more pronounced decreases in gray matter and total brain volume[2,88,89,99,100]. Reductions in volume of the hippocampus[12] and primary auditory cortex[34] have also been shown to correlate with illness duration. These brain changes have been posited to be due to the potentially progressive nature of the illness[2,76,98]. Although these structural changes do not necessarily correlate directly with progressive changes in cognitive functioning[101], some studies have reported correlations with worse performance on tests of executive functioning and worse symptoms over time[89,90,95]. Additionally, a longer duration of illness has been found to indicate unresponsiveness to treatment, which implies a more severe disease course[102].

MRI studies have found that decreases in gray and white matter in schizophrenia correlate with earlier age of onset and longer illness duration[99,100]; this may indicate that those who have an earlier onset exhibit more altered neuropathology[99]. A recent review put forth an argument that age at onset of schizophrenia coincided with age of brain maturation, which altered subsequent aging processes[103]. Those who have an earlier age of onset tend to have larger and more widespread cognitive deficits, greater impulsivity and a more severe course of illness, compared to those who have adult- or late-onset schizophrenia[104-106], although delusional conviction and poor insight were correlated with a later onset[107]. Cannabis use is often studied in relation to early onset, and both this and birth complications have been shown to predict early onset in males[108,109]. This relationship may also be found in females who carry a specific polymorphism of the BDNF gene[110].

A meta-analysis reviewing the gender difference in schizophrenia indicated earlier age of onset in males, as well as poorer social functioning and more severe negative symptoms than females[111-113]. However, a recent study found that the gender difference in age of onset may be much smaller than has been previously described, at a little over a one year difference in average age of onset between males and females[114]. This gender difference appeared to change as a function of the diagnostic tool used to diagnose the illness (ie. DSM vs ICD), and may be due to the different criteria for schizophrenia contained therein[114]. There have also been interactions between duration of illness and gender: size of the hippocampus related to duration of illness in females but not males[115]. Such gender and illness duration interactions will be discussed in chapter two of the current work.

Additionally, a study looking at asymmetry and age of onset noted that there was a gender difference in asymmetry in schizophrenia, and that this gender difference was opposite to that seen in controls[116]: a frontal lobe asymmetry where the left hemisphere was larger than the right hemisphere correlated with a later age of onset, especially in males[116]. This may indicate that a leftward asymmetry confers an advantage in males. Asymmetry effects will be discussed in relation to regional changes in chapter one.

The effect of medication on brain changes in schizophrenia is often difficult to quantify, due to lack of data available, heterogeneity in medication type, and effects of cumulative lifetime dosage. A meta-analysis of the literature has found that, in general, antipsychotics serve to reduce brain volume and increase ventricular volume[117], although increases in volume of the basal ganglia are also typical[118]; however, it remains unclear whether it is the medication itself having this effect or whether the drugs are interfering with the natural progression of the disease[119]. A large study looking at the effects of medication on cortical thickness found that there was no significant effect in either older or younger patients[120], although it has been shown that medication causes changes in the brain, especially an increase in volume of the caudate[89]. It cannot be ruled out that progressive changes in schizophrenia are due to medication effects, although progressive changes in brain volume have been seen in first-episode patients with very little antipsychotic exposure[88]. Different effects have also been seen between the two classes of antipsychotic medication, typicals and atypicals. Typical antipsychotics were first introduced in the 1950s, and have an effect by blocking dopamine receptors, while the more recently introduced atypical antipsychotics have more effect on serotonin receptors[118]. Brain changes have been shown to be reduced in patients using atypical antipsychotics compared to typicals[93]. Even the regions that are affected by these different classes are not the same: typical antipsychotics affect the basal ganglia and

regions in the frontal and temporal lobes preferentially, while atypical antipsychotics appear to have the most effect on the thalami[118]. Although medication information was not available for all of the cohorts in the current work, medication effects, as well as age of onset and illness duration, will be discussed in relation to the findings from each study.

Current Study

As has been presented here, there are many pieces that contribute to understanding the alterations that occur in brain function and structure in schizophrenia. In order to create a complete picture of these changes, it is imperative to not only pay attention to the previous research that has occurred, but also to critically appraise this research in order to identify areas that require additional study or supportive documentation. Only by using all of the tools and resources to study this disorder will we fully be able to appreciate and understand the range of the changes that occur in schizophrenia. This body of work will contribute to the existing literature by examining the differences seen in schizophrenia at three levels of analysis: first, regional gray matter differences between patients and controls will be examined using DTI and image analysis techniques. This is a novel application of a robust and frequently used method. Second, an examination of the neuronal architecture will be undertaken using a meta-analytical approach, in order to survey the established literature and draw conclusions from a large body of studies. Third, the microstructure will be studied in detail in the inferior parietal lobe, an area that is rapidly becoming a 'hot' topic of investigation, yet is still relatively understudied. These three studies will contribute to the literature by providing a critical summary of and support for previous literature, as

well as performing original research by using different techniques to evaluate changes in schizophrenia.

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Chapter One: Anisotropy and diffusivity in ten regions in the cerebral cortex

Introduction

Recently, diffusion tensor imaging (DTI) has become more common as a technique for investigating brain differences in schizophrenia: it allows for a minute (voxel-voxel) investigation of the diffusion in any given area in the brain. Values such as fractional anisotropy and mean diffusivity, among others, can be extracted from such an investigation, and these have been used to make inferences about the underlying cortical tracts. These values can then be correlated with various clinical and demographic measures. Studies using DTI to measure fractional anisotropy have largely reported decreases in schizophrenia[1], often in the left hemisphere of the frontal lobes[2-8] and temporal lobes[8-13]. However, there have also been reports of increased anisotropy in schizophrenia, especially in those who experience auditory hallucinations[14-16]. Increases in mean diffusivity (MD) have been reported in the parahippocampal gyrus and the insula[17], as well as the superior temporal gyrus[18]; however, measures of anisotropy are more commonly reported. Most, if not all, of the DTI studies that have been performed in schizophrenia have explored the white matter; this provides important information about tracts and connectivity in schizophrenia, and how they are related to functional networks and deficits. However, neglecting the gray matter in this regard is missing out on potentially informative analyses.

Although the gray matter is not traditionally though to have 'tracts' in the same way that the white matter does, it has an underlying structure that is potentially altered in schizophrenia[19]. Minicolumns have been reported to be the smallest functioning unit of the cortex[20], and, as such, investigating these structures in schizophrenia are able to

reveal important details about microstructure, with implications for the level of neuropil and neuron density, important issues in microanatomical work in schizophrenia. Although histological studies are still able to provide more detailed information on these structures, studying them in an *in vivo* model has advantages, including being able to study functional correlations, as well as being able to study many regions of interest (ROIs) at the same time.

In the current study, we have chosen to analyze diffusion in gray matter in schizophrenia in ten different brain regions, using an automatic segmentation method. Additionally, an innovative manual segmentation method, which is able to output additional values such as cortical thickness and angle of diffusion, was validated against the automatic method in a subset of cases. Fractional anisotropy and mean diffusivity, the outputs from the automatic segmentation, were correlated with demographic variables (age and gender, as well as age of onset, illness duration and medication, if applicable), as well as performance on several scales, including the Hayling Sentence Completion Task, the Positive and Negative Syndrome Scale, and scores for verbal IQ and full-scale IQ.

Regions of Interest

The ten regions that were analyzed included Broadmann areas (BA) 9, 11, 17, 39, 40 and 41, as well as the fusiform cortex, parahippocampal cortex, planum temporale, and precentral gyrus.

BA 9, which corresponds to the dorsolateral prefrontal cortex, is known to be involved in executive functioning processes, such as planning and organization. The orbitofrontal cortex, BA 11, is involved in decision-making; these are processes that are

disrupted in schizophrenia. Much of the research on schizophrenia has tended to focus on the frontal cortex, and these areas in particular, as a result. Studies have found increased density of interstitial white matter neurons in these regions, which is hypothesized to relate to the failure of inhibitory neurons to migrate appropriately in development[21-23]. Previous studies have found that the density of genes implicated in schizophrenia, as well as genes for markers of interneurons, have been reduced specifically in BA 9[24]; reduced gray matter has been found in BA 11, which correlated with scores on intelligence in schizophrenia but not in controls[25].

BA 17, the primary visual cortex, was initially chosen to act as a control region in this study, as used in other studies[26,27]; no changes in density, distribution or size of neurons in BA 17 were found in studies where it was used as a control region. However, recent studies have shown that there may indeed be changes in this region in schizophrenia: there are reports of deficits in early visual processing in schizophrenia[28], as well as increased gyrification in the visual cortex[29]. Additionally, Dorph-Petersen et al[30] found decreased neuron number and volume specifically in BA 17.

BA 39 (angular gyrus) and BA 40 (supramarginal gyrus) together make up the inferior parietal lobe; although it is common to group these areas together, based on my prior neuroanatomical investigations into these areas (described in chapter three), they were kept as two separate regions. The inferior parietal lobe is known to be the site of functional differences in schizophrenia, particularly in sensory integration and sense of self[31]. A recent meta-analysis of the literature has indicated that changes in schizophrenia may start in the parietal lobe and then migrate frontwards[32]; additionally, a study on functional circuitry has found that the key circuit that is affected in schizophrenia primarily involves parietal regions[33].

BA 41, otherwise known as Heschl's gyrus, is the site of the primary auditory cortex. As such, it is an important area to study in schizophrenia: severity of auditory hallucinations has been found to associate strongly with this area[34]. The planum temporale is located close to BA 41, and is involved in processes to do with language. It is also one of the most asymmetrical structures in the brain, and findings have shown that this asymmetry is decreased in schizophrenia[35,36]. There is a reduction in inhibitory neurons in the planum temporale in schizophrenia[37], and studies have found decreases in volume in both Heschl's gyrus and the planum temporale in first-episode schizophrenia, particularly in the left hemisphere[38,39].

The fusiform cortex has traditionally been known as the location of the fusiform face area, an area important for facial and person recognition. A reduction in minicolumn spacing, a unit of microstructure, as well as reduced neuron density, has been found in the fusiform cortex in schizophrenia[40]. The parahippocampal gyrus, similarly to the fusiform gyrus, is known for the 'parahippocampal place area'; this area is important for the encoding and recognition of scenes and places. Deactivation of this region has been found to occur before auditory hallucinations[41]. Reduced gray matter volume has been found in both the fusiform and parahippocampal gyrus in patients, specifically in the left hemisphere[42,43].

The precentral gyrus is known as the motor cortex; this is where motor activity originates. In schizophrenia, there are known abnormalities in movement such as dyskinesia and parkinsonism, and these have been found to occur in medication-naïve patients[44]. A recent study found that fractional anisotropy values correlated with activity in the right precentral gyrus in patients[3].

Given the plethora of changes and deficits seen in these regions in schizophrenia, they are viable candidates for a DTI analysis of cortical structure in the gray matter. Additionally, histological research in BA 41, planum temporale, fusiform cortex and BA 39 and BA 40 has already been performed in this lab, and including these regions in this DTI analysis was of particular interest.

Although there are other brain regions that are of interest in schizophrenia, the decision was made to focus on these 10 as opposed to other cortical structures, given their involvement in the pathogenesis and maintenance of schizophrenia, as well as *a priori* hypotheses of involvement. Subcortical structures were not chosen as this novel use of DTI in gray matter is meant to look at the cortical microstructure underlying the gray matter.

Clinical Variables

It has been suggested that IQ in schizophrenia correlates with decreased fractional anisotropy values in frontal white matter and also with negative symptoms[45]. Studies of IQ in schizophrenia have looked both at current and premorbid IQ, and have found that there is a relationship between IQ at psychosis onset and later functioning: lower IQ at psychosis onset predicts more symptoms and worse outcomes later in the illness than those with a higher IQ[46]. Scores on verbal as well as full-scale IQ were examined in relation to anisotropy, age of onset, illness duration, and scores on the Positive and Negative Syndrome Scale (PANSS) in the current study.

Studies examining performance on the Hayling Sentence Completion Task have shown that schizophrenia patients perform significantly worse than both healthy

controls and bipolar patients, signifying that the executive functioning deficits revealed by this task are a particular aspect of schizophrenia[47]. However, performance has been shown to improve over time[48].

Those who have an earlier age of onset tend to have larger and more widespread cognitive deficits, compared to those who have adult- or late-onset schizophrenia[49], and MRI studies have found that decreases in gray and white matter in schizophrenia correlate with earlier age of onset and longer illness duration[50,51]. A meta-analysis reviewing the gender difference in schizophrenia indicated earlier age of onset in males, as well as poorer social functioning and more severe negative symptoms than females[52]. However, another recent study found that this gender difference is much smaller than has been previously described, at a little over a one year difference in average age of onset between males and females[53]; this gender difference appeared to change as a function of the diagnostic tool used to diagnose the illness (ie. DSM vs ICD).

The effect of medication on brain changes in schizophrenia is often difficult to quantify, due to lack of data available, heterogeneity in medication type, and effects of cumulative lifetime dosage; however, a large study looking at the effects of medication on cortical thickness found that there was no significant effect in either older or younger patients[54]. This same study also found that there was no effect of age on cortical thickness; however, there have been reports of altered aging effects in schizophrenia, especially on the microstructure[40,55].

Methods

This study was carried out on data obtained from the CAFLIP (Cerebral Asymmetry and Functional Imaging in Psychosis) study, led by primary investigator Dr.

T.J. Crow, University of Oxford. The subjects were recruited by psychiatrists from a clinical population in and around the Oxford area. Overall, DTI and demographic data were available for 110 subjects; of these, 54 were selected for analysis, based on suitability of matching patients to controls. In order to control for age and gender, healthy controls were matched to patients if (i) they matched exactly on age and gender; (ii) they matched on age but not gender; or (iii) they matched on gender and age within one year. These 54 were made up of 27 schizophrenia patients and 27 matched controls. 26 subjects (13 controls and 13 patients) were matched on exact age and gender; 10 subjects (5 controls and 5 patients) were matched on exact age but not gender; 10 subjects (5 controls and 5 patients) were matched on gender, and age within one year; and 8 subjects (4 controls and 4 patients) were matched on age within one year but not gender. Although there were other subjects available to be analyzed, as we were investigating age interactions, it was decided that only those subjects that were well-matched would be selected for analysis. Scores were available from a standard verbal IQ test and a general IQ test, as well as from the Hayling Sentence Completion Task. For patients, data on age of onset and length of illness was obtained, as well as scores on the Positive and Negative Syndrome Scale (PANSS).

The demographic and clinical data for each subject was obtained from Dr. S.A. Chance, University of Oxford (see Tables 1 and 2); the DTI scan data was obtained from the online file server for the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB). This is accessed using the FMRIB Software Library (FSL[56-58]), an image-analysis software suite developed by FMRIB. FSL requires a virtual machine to be able to be run on a Windows computer; FMRIB recommends the VMWare Player, and this was obtained via the VMWare Player website

(http://www.vmware.com/products/player/). The DTI scans were also analyzed using these software programs.

Table 1. Demographic data for subjects included in analysis

Subjects	n	Gender	Age	Hay Total	Verbal IQ	FS IQ
Controls	27	18M : 9F	28.48	6.10	26.81	121.81
Patients	27	17M : 10F	28.52	5.05	21.74	107.42

* All values are mean values (except for gender)
* n=number of subjects; Hay Total=score on the Hayling Sentence Completion Task; Verbal IQ=score on a standard verbal IQ test; FS IQ=scores on a general IQ test
* n=19 for Hay Total, verbal IQ and FS IQ in patients; n=21 for Hay Total, verbal IQ and FS IQ in controls
* Age is measured in years; other values are scores on respective tests

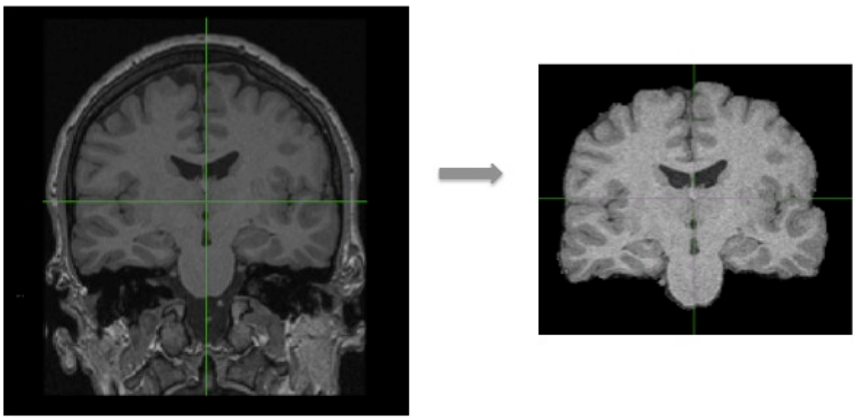
Table 2. Clinical variables, patients

Subjects	Age of onset	Length of illness	Chlorpromazine equivalents	PANSS Pos	PANSS Neg	PANSS Dis	PANSS Total
Patients	22.00	6.63	457.25	17.72	19.78	12.06	94.50

* n=number of subjects; all values are mean values
* n=19 for age of onset and length of illness; n=16 for chlorpromazine equivalents; n=18 for PANSS Pos, PANSS Neg, PANSS Dis and PANSS Total
*Age of onset and length of illness are measured in years; chlorpromazine equivalents are measured in mg; PANSS values are scores for each subset of questions on the Positive and Negative Syndrome Scale
* Pos=Positive; Neg=Negative; Dis=Disordered

Two scans were obtained for each patient; the first was the base structural scan, at a resolution of 1 mm^3. The second was a scan of diffusion data, and this was at a resolution of 2.5 mm^3. The structural scan was a full head image; therefore, before any analyses could begin, the skull portion of the image had to be removed, using the Brain Extraction Tool[59] in FSL (see Figure 1).

Figure 1. Before and after running the BET program



The image on the left is a base structural scan for one subject; the image on the right is the brain-extracted image of the same subject.

Automatic Segmentation

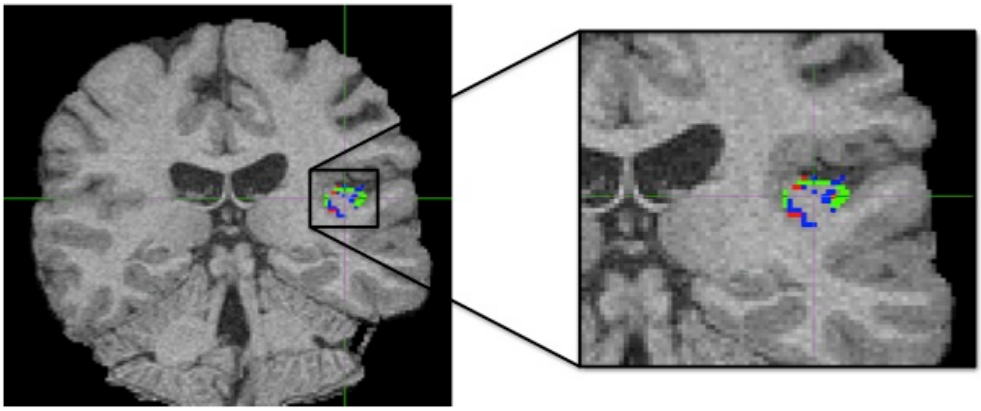
Ten different brain areas were examined: BA 9, BA 11, BA 17, BA 39, BA 40, BA 41, fusiform gyrus, parahippocampal gyrus, planum temporale, and precentral gyrus. Nine of these areas were selected because of their noted involvement in the pathology of schizophrenia (BA 9, BA 11, BA 39, BA 40, BA 41, fusiform gyrus, parahippocampal gyrus, planum temporale, and precentral gyrus). BA 17 was chosen as a control region.

Three different brain atlases were used to delineate these brain areas: the Juelich Histological Atlas, the Talairach Daemon Labels, and the Harvard-Oxford Cortical Structural Atlas. These were pre-loaded with the FSL program, and were available for viewing via the image viewer, FSLview. These three atlases were chosen after careful examination of the borders and coverage over the 10 selected structures. The Juelich Atlas was used to delineate BA 39, BA 41, and the precentral gyrus; the Talairach Labels were used for the parahippocampal gyrus, BA 17, BA 9 and BA 11. The Harvard-Oxford Atlas was used to delineate the fusiform gyrus and the planum temporale, and a combined mask from the Juelich and Talairach was used to delineate BA 40, as neither mask fully captured the area. Each of these three atlases had their strengths and weaknesses; for example, the Talairach Labels used BA 39 and BA 40 as signifiers for the inferior parietal lobe, whereas the Juelich Atlas used the more unusual Von Economo classification, PE, PF, PG, etc. Therefore for this region, the Juelich Atlas had more masks that potentially covered the same (or a similar) region as the Talairach. Additionally, many of the labels and/or the segmentations that were built-in with these automated masks were not necessarily correct; therefore, in order to create masks of the desired regions of interest, knowledge of anatomical regions and boundaries was used when selecting and combining masks. Since masks from different atlases were used, special care was taken when examining for overlap between the masks on the standard brain;

scripts were built-in to the warping procedure to remove any potential overlap once the masks had warped onto the individual brains. This was particularly important for neighboring regions, for example BA 41 and PT, or BA 39 and BA 40.

Individual masks for each structure were extracted from the respective atlas at the maximum threshold level possible; higher thresholding indicated a more accurate mask (see Figure 2). Each mask was split into a left and a right hemisphere, in order to facilitate analysis of hemispheric differences; this was only necessary for the masks from the Harvard-Oxford Atlas, as the other atlases had already divided the masks into hemispheres.

Figure 2. Examples of different levels of thresholding



The mask for region BA 41 is used as an example to illustrate the different levels of thresholding: the gray matter mask in red represents a mask of 50% gray matter; ie. voxels are included in the mask if they are at least 50% gray matter; the blue mask is a mask of 80% gray matter, and the green mask is a mask of 100% gray matter. The 100% gray matter thresholding level was used in the current study.

The masks for the ten areas were examined on the standard brain (Montreal Neurological Institute, or MNI, brain; resolution of $1mm^3$) for any overlapping between regions; this overlap was removed using Fslmaths. There were also several possible overlaps between masks that had very close borders but were not actually overlapping on the standard brain; although these were not an issue on the standard brain, due to the variation in brain sizes and shapes, these may have become issues when analyzing

individual subjects. Therefore, these overlaps were addressed using the scripted procedure to transfer the masks from the standard brain to each individual subject brain: in each individual brain, any overlap was removed via commands written in this script. The various scripts that were used were obtained from a colleague, and were based on the outputs printed by the relevant image analysis tools. These scripts automated the analysis process and made it possible to analyze several subjects at one time.

Each individual had an original structural scan (with the brain and skull), which was referred to as the whole-brain image; the image with just the brain was referred to as the structural image. The first script that was used was referred to as the 'registration' script. This script was used to register each individual structural image and diffusion image to the standard brain image, and vice versa. This was necessary in order to have the diffusion data mapped correctly onto the structural image, as well as to map the masks from the standard brain onto the structural brain. This was done by creating six matrix files: a diffusion to structural, structural to diffusion, structural to standard, standard to structural, diffusion to standard, and standard to diffusion. These files were used to resolve the difference in resolution between the diffusion, standard, and structural images.

The second script, called the 'warping' script, was used to map the diffusion data and masks to the structural image; this is called warping, and was done using the matrix files created in the registration section. Each of the masks was applied to the structural image, as was the diffusion data. The masks were thresholded to 100%, meaning that the only voxels that were included in the mask were 100% gray matter; this was known as the gray matter mask. Any potential overlap between individual masks was removed

at this point. The output from this 'warping' step included each individual mask, mapped onto the structural scan, plus separate gray matter masks which were adjusted for the amount of gray matter in each individual brain. Diffusion data, including fractional anisotropy and mean diffusivity, were applied to the structural scan and to each mask.

Finally, the third script was the 'summarize' script. This was used to extract values from the diffusion data for each mask. The output from this script was a value for fractional anisotropy (FA) and mean diffusivity (MD) for each regional mask. Additionally, a script was used to obtain volumes for the whole brain, gray matter and white matter, as well as volumes for each region. Statistical analyses were carried out using these values.

Manual Segmentation

After preliminary analysis was carried out on the FA and MD values, it was decided that a manual segmentation process should be carried out on a small subset of subjects. The manual segmentation process, called CHIPS, was a new procedure initiated by FMRIB; it was alleged to be more accurate than the automatic segmentation, but had not been validated by outside groups, and was substantially more time-consuming. For these reasons, this procedure was carried out on a small subset of subjects (ten patients and ten controls). These subjects were selected as follows: a scatter-plot was drawn for values of FA and MD for each region, and a normal distribution curve was approximated for each region, for each of FA and MD. Subjects that had FA and MD values that were in line with this distribution curve were compared between regions, and the subjects that most closely approximated this curve between all regions were selected. Three regions of interest (BA 9, BA 39, and BA 41) were chosen, based on preliminary data from the automatic segmentation study; these regions of interest (ROI) were chosen because they

highlighted differences between patients and controls. Additionally, only the left hemisphere of each brain was analyzed, as changes in asymmetry are more likely to be due to changes in the left hemisphere[60]; therefore, any differences that appear in schizophrenia may be amplified here.

Table 3. Demographic data for CHIPS cohort

Subjects	n	Gender	Age	Hay Total	Verbal IQ	FS IQ
Controls	10	8M:2F	26.20	4.63	26.75	121.63
Patients	10	8M:2F	29.90	5.30	21.10	102.80

* All values are mean values (except for gender)
* n=number of subjects; Hay Total=score on the Hayling Sentence Completion Task; Verbal IQ=score on a standard verbal IQ test; FS IQ=scores on a general IQ test
* n=8 for Hay Total, verbal IQ and FS IQ in controls; all other n=10
* Age is measured in years; other values are scores on respective tests

Table 4. Clinical variables for CHIPS cohort

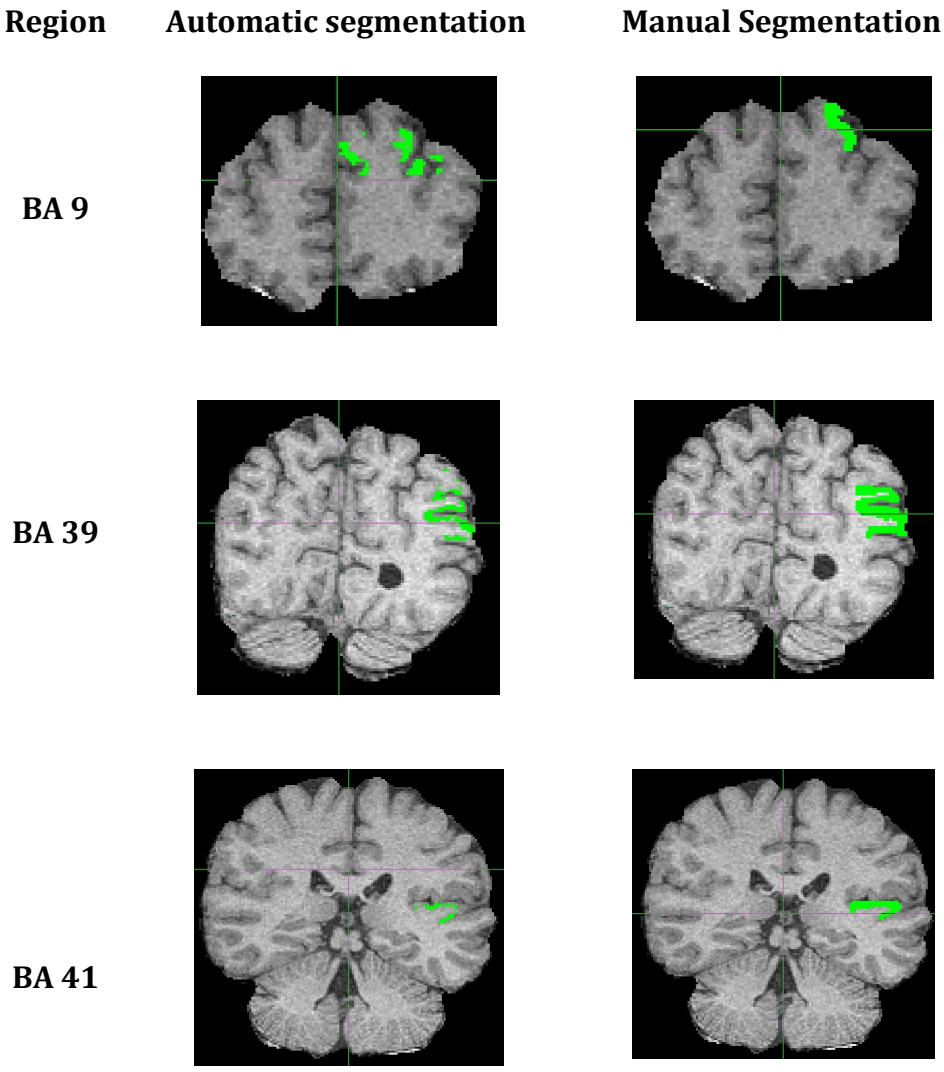
Subjects	Age of onset	Length of illness	Chlorpromazine equivalents	PANSS Pos	PANSS Neg	PANSS Dis	PANSS Total
Patients	23.90	6.00	481.25	16.90	19.20	12.00	90.60

* n=8 for chlorpromazine equivalents; all other n=10
* All values are mean values
*Age of onset and length of illness are measured in years; chlorpromazine equivalents are measured in mg; PANSS values are scores for each subset of questions on the Positive and Negative Syndrome Scale
* Pos=Positive; Neg=Negative; Dis=Disordered

For each brain region in each subject, the middle 15 slices of the mask were selected for further analysis; the 15 slices that were selected were chosen as they were most representative of the chosen region, based on visual analysis. Using the brain atlases, the gray matter mask from the automatic segmentation, and knowledge of neuroanatomy, the ROI was outlined and colored in fully in these 15 slices. This was done in order to obtain smooth edges from one slice to the next, and was a crucial step for the CHIPS program to work. This was first done using the coronal view, and then was verified in both the sagittal and axial views. It was apparent that this manual segmenting of each region was slightly different to the automatic segmentation (Figure 3), likely due

to the decision to use the 100% gray matter masks in the automatic segmentation (see Figure 2).

Figure 3. Masks from the automatic segmentation and the manual segmentation, in BA 9, BA 39, and BA 41 (coronal view)



Once the ROI had been colored in, the outside edge (border between gray matter and cerebrospinal fluid) and inside edge (border between gray matter and white matter) were identified. The CHIPS procedure worked to calculate values in between these borders, which was why smooth edges between the slices was crucial. The last slice on either end, as well as the border voxels on either edge, were not included in the value calculation. This manual procedure was repeated for each ROI in each subject; the

CHIPS script was then run, and a summarize script was used to obtain values. CHIPS was able to output more values than the automatic segmentation, including V1 angle (angle of diffusion from the vertical), V1 dot product (dot product of the angle: cosine of the angle), scaled V1 parallel (proportion of the principal diffusion direction that is parallel to the vertical), and scaled V1 perpendicular (proportion of the principal diffusion direction that is perpendicular to the vertical). FA and MD values were also available, and were used to validate the CHIPS method against the automatic method. A third value for FA and MD was also calculated; this value, known as FA intermediary and MD intermediary, used the manual masks created in the CHIPS procedure but included all border areas in the value calculation. This intermediary value was calculated to examine differences in the CHIPS calculation from the automatic calculation.

Results

Statistical Analysis

Values for fractional anisotropy (FA), mean diffusivity (MD), and regional volume were calculated for each hemisphere in each region, for each subject. The values for the hemispheres were then averaged within each region in each subject (see Table 5 for FA and MD values, and Table 6 for volumetric values).

Table 5. Results from automatic segmentation

Measure	FA		FA: Asymmetry		MD		MD: Asymmetry	
	Controls	SZ	Controls	SZ	Controls	SZ	Controls	SZ
BA 9	0.102	0.107	-0.0240	-0.0653	0.000854	0.000951	-0.0240	-0.0259
BA 11	0.132	0.131	0.0597	0.0298	0.000889	0.000930	-0.0384	-0.0226
BA 17	0.117	0.109	-0.0688	-0.0170	0.000967	0.001040	0.0693	0.0713
BA 39	0.108	0.110	-0.0111	-0.0205	0.000923	0.000954	-0.0107	-0.0096
BA 40	0.109	0.111	-0.0119	0.0012	0.000953	0.000977	-0.0185	-0.0141
BA 41	0.122	0.120	0.0912	0.0892	0.000979	0.001020	0.0351	0.0250
Fus	0.148	0.131	0.0767	0.0694	0.000864	0.000920	0.0457	-0.0109
Para	0.143	0.139	0.0347	-0.0538	0.001090	0.001090	-0.1030	-0.0817
PT	0.160	0.149	0.0271	0.0244	0.000924	0.000955	0.0143	0.0080
PCG	0.128	0.124	0.0754	0.0649	0.000932	0.000955	0.00842	0.0139

*All values are mean values

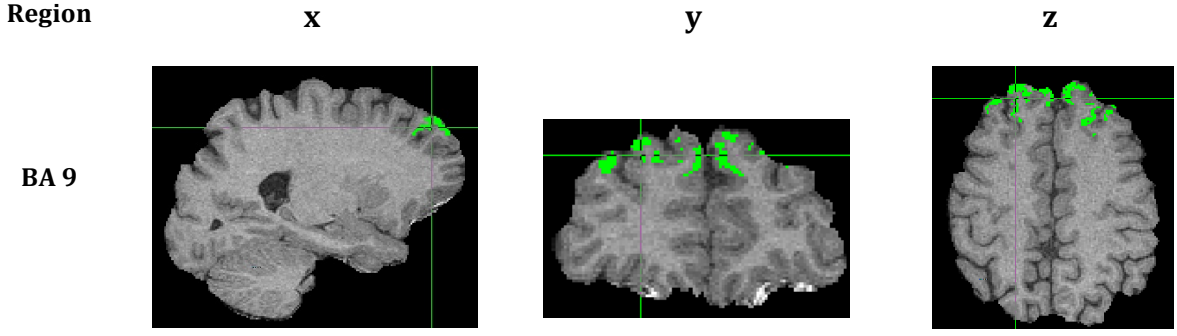
Due to the large amount of data collected, the decision was made to analyze only the averaged data, ie. only the mean values for each region for each subject, and not the separate hemispheric values. In order to validate this, repeated-measures ANOVAs were computed between hemispheric values and the mean value for FA, MD, and regional volume, separately for controls and patients. In patients, there were no significant differences between the left hemisphere value and the mean value, or the right hemisphere value and the mean value, for all of FA, MD and the regional volumes. For controls, there were no significant differences between the right hemisphere value and the mean value for FA, MD, or regional volume; there were no significant differences between the left hemisphere and the mean value for MD or regional volume. However, there was a significant difference between the left hemisphere value and the mean value for FA in controls (df=1, F=4.754, p=0.030); post-hoc tests were computed to look at the differences by region. The only regions that significantly differed between the value for the left hemisphere and the mean value were BA 41 (paired-samples t-test: t=-2.135, df=26, p=0.042) and PCG (related-samples Wilcoxon Signed Rank Test: p=0.006). However, both of these regions were significantly correlated between the left hemisphere value and the mean value (BA 41: p=0.000; PCG: p=0.000). Given that there were only two regions that were significantly different, it was decided that all further analysis should proceed using only the mean values; however, caution should be taken when interpreting results from BA 41 and PCG in FA in controls.

Mean values were analyzed for possible differences between the diagnostic groups, as was a measure of asymmetry for each region. This value of asymmetry was calculated as follows: the value for the left hemisphere was subtracted from the value for the right hemisphere, and divided by the mean value (R-L/mean). This gives a way to

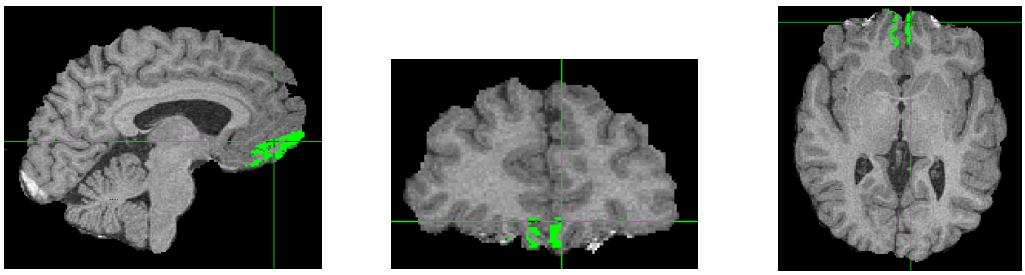
look at the relative difference between hemispheres, ie. the asymmetry, for each region, and analyze this between subjects and diagnostic groups.

Ten brain regions were included in the following analyses: Brodmann Areas (BA) 9, 11, 17, 39, 40 and 41, as well as the fusiform gyrus (Fus), parahippocampal gyrus (Para), planum temporale (PT), and precentral gyrus (PCG). The gray matter masks for these regions are available in Figure 4. Data was also available for performance on a variety of clinical scales, including tests of verbal IQ and full-scale IQ, as well as the Haylings Sentence Completion Task. For this task, total values (Hay Total) were calculated for each subject and subjected to statistical analyses; for the IQ tests, verbal IQ and full-scale IQ were subject to analyses. Additionally, illness-specific variables such as age of onset, duration of illness, medication (as measured by chlorpromazine equivalents), and performance on the Positive and Negative Syndrome Scale (PANSS) were included in the statistical analyses. Performance on the PANSS was indicated by scores for the negative subtotal (PANSS Neg), the positive subtotal (PANSS Pos), the disorganized subtotal (PANSS Dis), and the overall total (PANSS Total). These subtotals respectively group together scores on negative symptoms, positive symptoms, and disorganized symptoms. Age and gender data were also included in the analyses. SPSS (version 20) was used for all analyses.

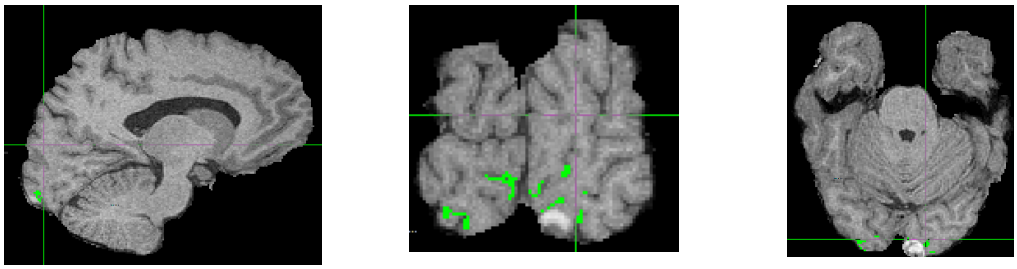
Figure 4. Gray matter masks of regions of interest, automatic segmentation



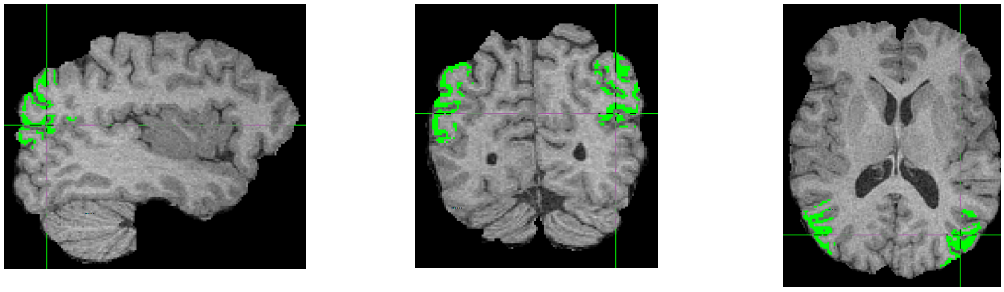
BA 11



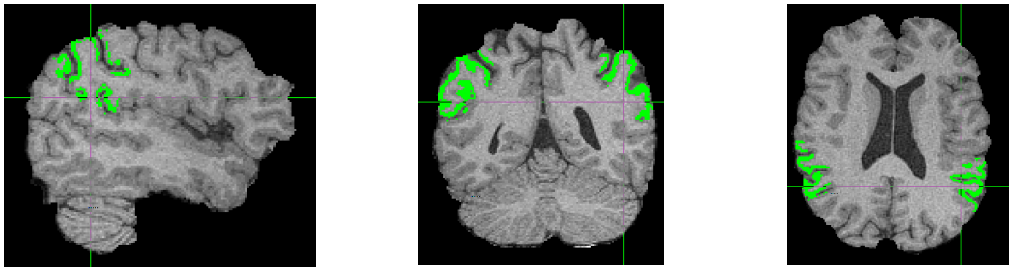
BA 17



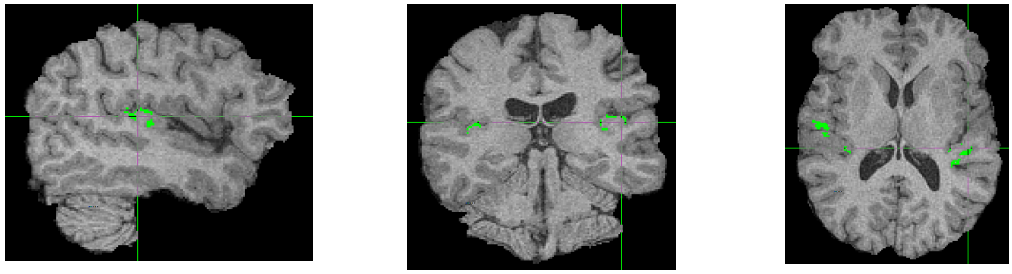
BA 39



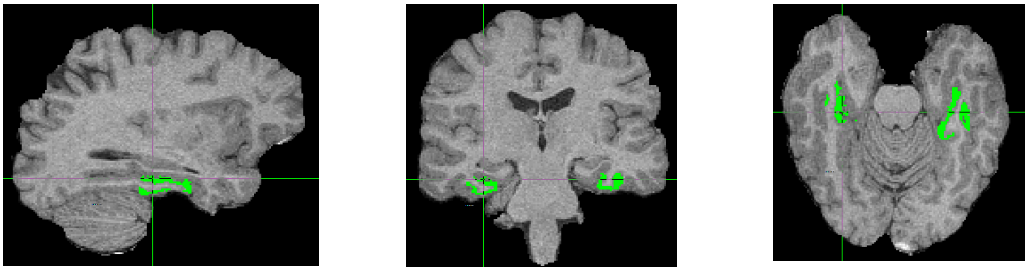
BA 40

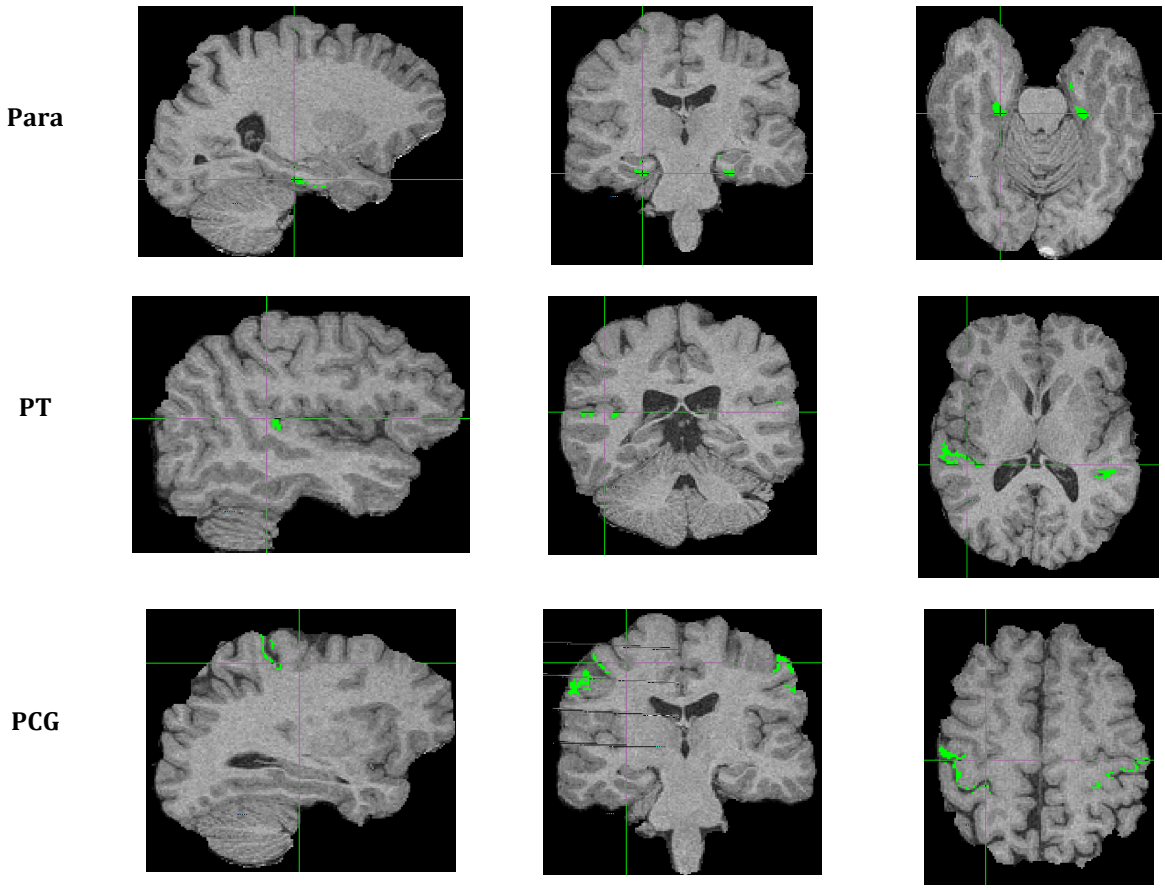


BA 41



Fus

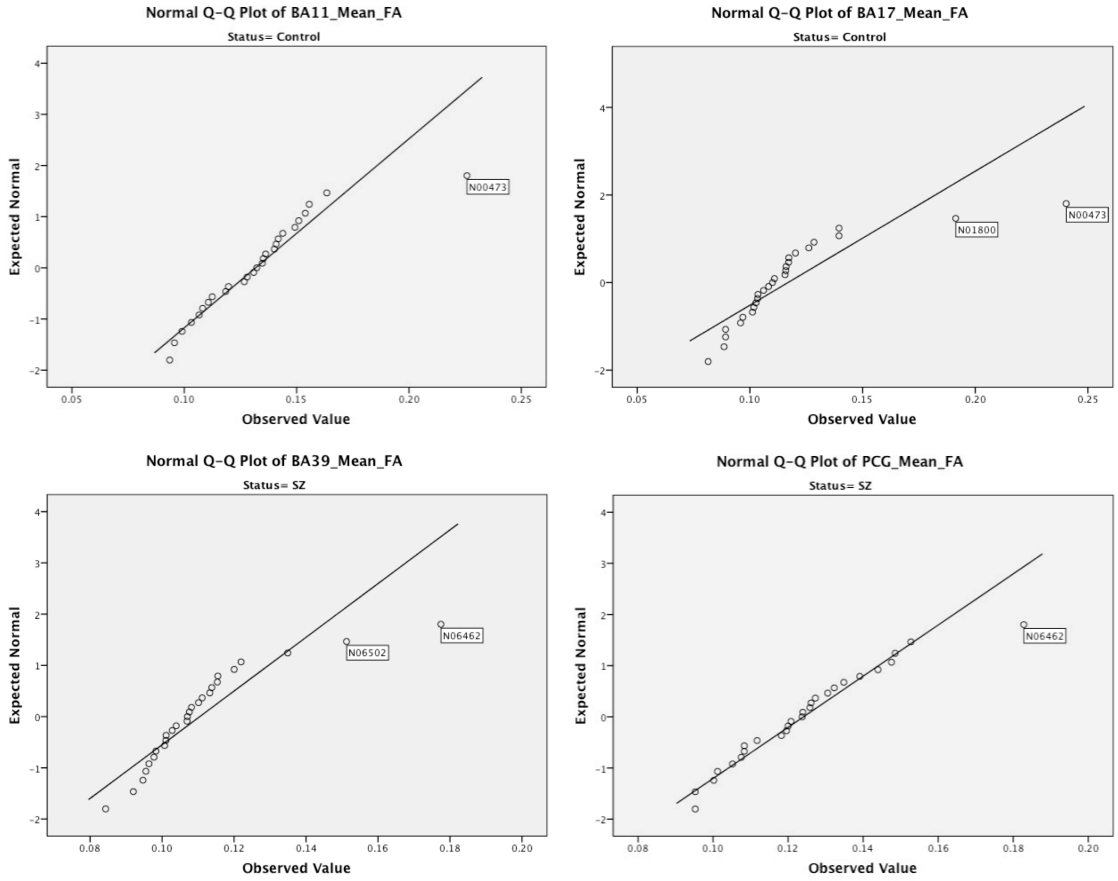




Outliers

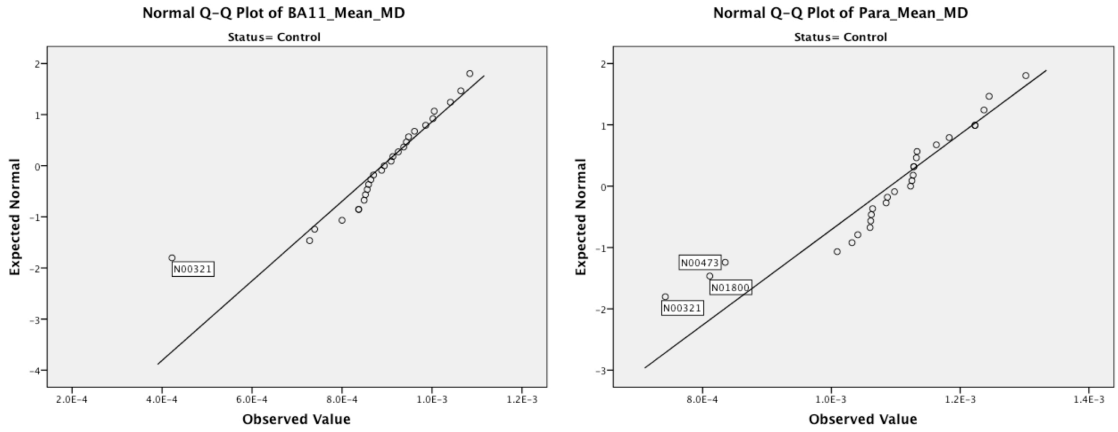
Normal Q-Q plots were created for each of the regions on measures of FA and MD. From visual analysis of these, it was clear that there were individual values that did not fall in line with the rest of the distribution and may have been considered outliers (Figures 5 and 6). However, there were a substantial number of these, and they were not all the same subject; given this, and the fact that the sample size was small (n=27 for both controls and patients), it was decided to include all subjects in the following analyses. If the outliers were removed from the analysis, it would have had to be done on a region-by-region basis for both controls and patients, which would have greatly reduced statistical power. Although some of the outliers are similar between the regions in both the control and patient groups, removing all of the outliers would have decreased the sample size by a large proportion, thus decreasing statistical power.

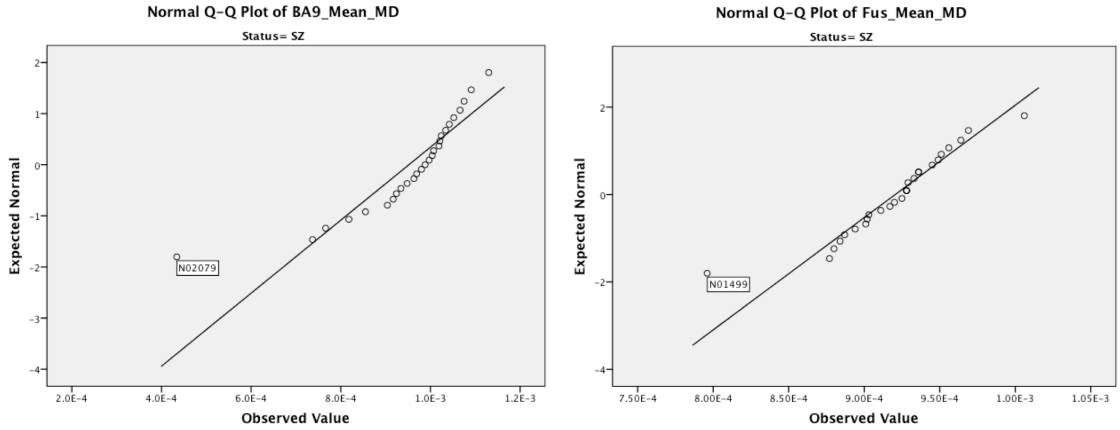
Figure 5. Normal Q-Q plots for FA



** These particular Q-Q plots were chosen as they were representative of all the regions; labels indicate subject identifier for outliers.*

Figure 6. Normal Q-Q plots for MD





** These particular Q-Q plots were chosen as they were representative of all the regions; labels indicate subject identifier for outliers.*

Tests of Normality

The Shapiro-Wilk test was used to test for normality, because the sample size for each diagnostic category was relatively small (n=27); the Shapiro-Wilk test has been optimized for small sample sizes. Those variables that were significant on a test of normality, as evidenced by the Shapiro-Wilk test statistic, were considered to have failed the test of normality and were analyzed using nonparametic tests. Demographic and clinical variables, such as age, IQ, and performance on the PANSS, were also analyzed for normality. Table 1 in Appendix A lists the regions and variables that failed a test of normality; this is listed for the measures of FA and MD, regional volumes, and the demographic and clinical variables, as well as for variables included in the CHIPS procedure (Table 2 in Appendix A).

Whole-Brain Volumes

There were no significant differences between the diagnoses for any of the whole-brain volumes (gray matter volume, white matter volume and total brain volume).

Whole-brain volumes were correlated with FA in BA 17 in controls (gray matter volume: Spearman's rho=0.522, p=0.005, n=27; white matter volume: Spearman's rho=0.626, p=0.000, n=27; total brain volume: Spearman's rho=0.522, p=0.005, n=27) and in patients (gray matter volume: Spearman's rho=0.465, p=0.014, n=27; white matter volume: Spearman's rho=0.470, p=0.013, n=27; total brain volume: Spearman's rho=0.465, p=0.014, n=27).

Table 6. Volumetric values, main cohort

Measure	Controls	SZ
BA 9	2226.15	1959.46
BA 11	1237.83	1139.61
BA 17	623.67	654.21
BA 39	5707.99	5932.69
BA 40	10915.96	10831.83
BA 41	1097.32	1020.55
Fus	1970.14	1917.54
Para	457.62	437.87
PT	755.35	748.18
PCG	2436.50	2376.85
Whole-brain GM Volume	641780.57	629493.83
Whole-brain WM Volume	598981.01	596018.78
Whole-brain Total Volume	1283561.15	1258987.65

*All values are mean values; volume is mm^3

Regional Volumes

A repeated-measures ANOVA was performed on mean regional volume between diagnostic groups. There was no difference between controls and patients on a measure of mean regional volume, although there was a difference in volume between regions (df=1.744, F=1850.223, p=0.000); however, as there was no difference between regions between diagnoses, there was no further analysis of these differences.

Further repeated measures ANOVAs were also performed, using the demographic and clinical measures as covariates. There was no significant effect of diagnosis between subjects when any of these covariates were included, although age was a significant covariate (df=1, F=15.632, p=0.000).

FA

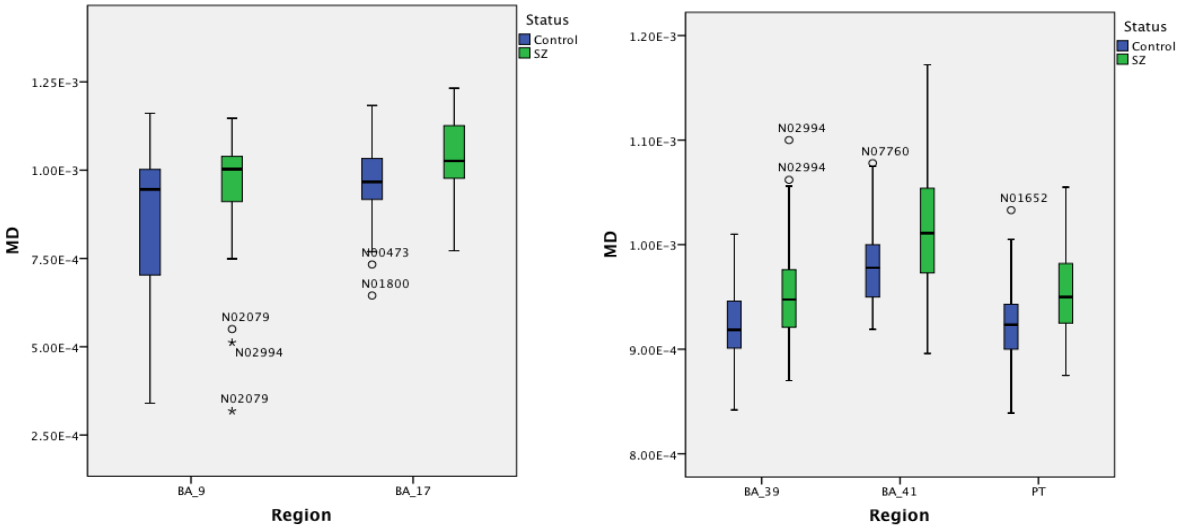
A repeated-measures ANOVA between diagnostic groups was performed on the mean FA data. There was no significant difference between patients and controls on a test of FA, although there was a difference in FA values between regions (F=31.019, df=2.512, p=0.000); however, as there was no difference between the regions between diagnoses, no further analysis of these differences was performed.

Further repeated measures ANOVAs were also performed, using the demographic and clinical measures as covariates. There was no significant effect of diagnosis between subjects when any of these covariates were included.

MD

A repeated-measures ANOVA was performed to look at the overall difference in MD between diagnostic groups. The difference between patients and controls on a measure of MD was significant (F=8.885, df=1, p=0.004), as was a difference in MD values between regions (F=29.136, df=3.532, p=0.000). Although an interaction between diagnosis and region was not significant, post-hoc tests indicated that values for MD were significantly different between patients and controls in BA 17 (t=-2.633, df=52, p=0.011), BA 39 (t=-3.147, df=52, p=0.003), BA 41 (t=-3.237, df=39.626, p=0.002), and PT (t=-3.409, df=52, p=0.001), as well as in BA 9 (p=0.028).

Figure 7. Differences in MD between patients and controls



** Regions have been shown on two graphs because of the difference in the scale of MD.*

Further repeated-measures ANOVAs were also performed, using the demographic and clinical measures as covariates. The only two covariates that increased the significance of the difference between patients and controls were age and gender; when age was included as a covariate, it was significantly different between subjects (F=10.193, df=1, p=0.002), as was diagnosis (F=10.396, df=1, p=0.002). Region was also found to be significantly different (F=4.743, df=3.614, p=0.002), as was an interaction between region and age (F=2.831, df=3.614, p=0.031). When gender was included (separately) as a covariate, it was found that diagnosis was significantly difference between subjects (F=9.402, df=1, p=0.003); region was also found to be significantly different (F=3.405, df=3.525, p=0.014).

FA symmetry

A repeated-measures ANOVA was performed between regions on values of asymmetry in FA between diagnostic groups. There was no significant effect of diagnosis between subjects, although there was a significant difference between regions (F=4.410, df=5.547, p=0.000); however, as there was no difference between the regions between diagnoses, no further analysis of these differences was performed.

Further repeated measures ANOVAs were also performed, using the demographic and clinical variables as covariates. There was no significant effect of diagnosis between subjects when any of these covariates were included.

MD Asymmetry

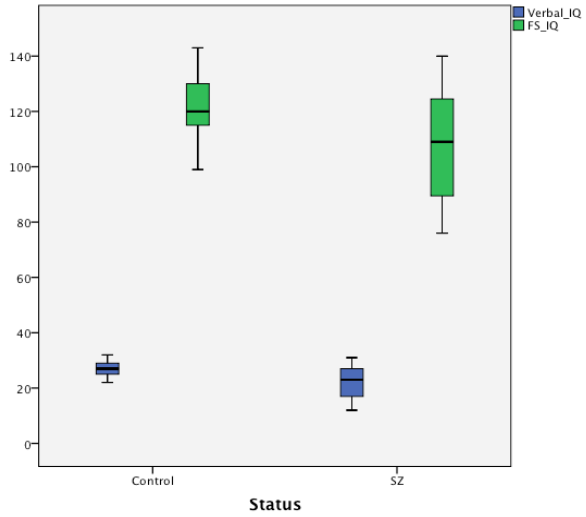
A repeated-measures ANOVA was performed between regions on values of asymmetry in MD between diagnostic groups. There was no significant difference in MD asymmetry values between the diagnoses, although there was a significant difference between regions (F=10.858, df=3.421, p=0.000); however, as there was no difference between the regions between diagnoses, no further analysis of these differences was performed.

Further repeated-measures ANOVAs were also performed, using the demographic and clinical variables as covariates. There was no significant effect of diagnosis between subjects when any of these covariates were included.

Demographic & Clinical Variables

A multivariate ANOVA was computed to look at the difference in age, gender, verbal IQ, full-scale IQ, and Hay Total between controls and patients. Verbal IQ (df=1, F=10.046, p=0.003) and full-scale IQ (df=1, F=7.410, p=0.010) were different between the diagnoses.

Figure 8. Verbal and full-scale IQ



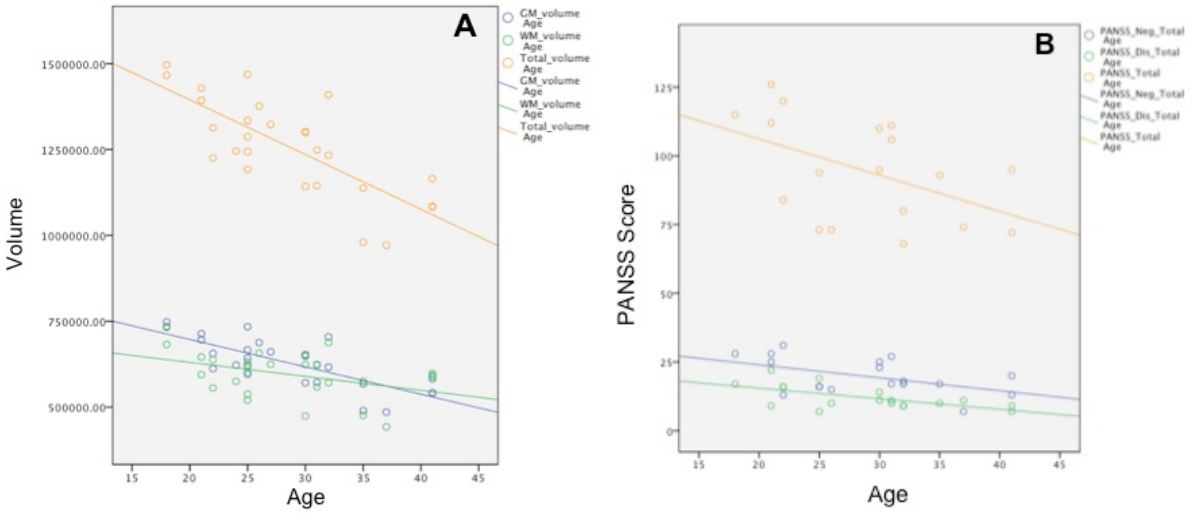
Differences between controls and patients on scores of IQ; blue is verbal IQ, and green is full-scale IQ. There is a decrease in both measures in patients.

Age

In controls, age was correlated with FA in BA 9 (Pearson correlation=0.464, p=0.015, n=27), BA 39 (Pearson correlation=0.464, p=0.015, n=27), Para (Spearman's rho=-0.425, p=0.027, n=27) and PCG (Pearson correlation=-0.450, p=0.019, n=27). Age was correlated with MD in BA 9 (Spearman's rho=0.596, p=0.001, n=27) and PCG (Spearman's rho=0.615, p=0.001, n=27).

In patients, age was correlated with age of onset (Spearman's rho=0.606, p=0.006, n=19) and length of illness (Spearman's rho=0.541, p=0.017, n=19). Age was also correlated with all measures of whole-brain volume (gray matter volume: Pearson correlation=-0.756; p=0.0000, n=27; white matter volume: Pearson correlation=-0.407, p=0.035, n=27; total volume: Pearson correlation=-0.756, p=0.000, n=27), as well as with scores from the negative PANSS (Pearson correlation=-0.501, p=0.034, n=18), disorganized PANSS (Pearson correlation=-0.616, p=0.007, n=18) and total PANSS (Pearson correlation: -0.486, p=0.041, n=18) (see Figure 9).

Figure 9. Relationships with age in patients



***A**: The relationship of age with measures of whole-brain volume; **B**: the relationship of age with scores on the PANSS*

In patients, age correlated with MD in PT (Pearson correlation=0.400, p=0.039, n=27) and PCG (Pearson correlation=0.448, p=0.019, n=27).

IQ and Semantic Measures

In controls, verbal IQ was correlated with the whole-brain volume measures (gray matter volume: Pearson correlation=0.516, p=0.017, n=21; white matter volume: Pearson correlation=0.462, p=0.035, n=21; total volume: Pearson correlation=0.516, p=0.017, n=21). Hay Total was negatively correlated with both gray matter volume and total volume (for both, Spearman's rho=-0.487, p=0.025, n=21). Hay Total was also correlated with age (Spearman's rho=0.462, p=0.035, n=21).

Verbal IQ and FS IQ were correlated with MD in BA 39 (verbal IQ: Pearson correlation=0.492, p=0.024, n=21; FS IQ: Pearson correlation=0.460, p=0.036, n=21) and BA 40 (verbal IQ: Pearson correlation=0.519, p=0.016, n=21; FS IQ: Pearson correlation=0.560, p=0.008, n=21). FS IQ was correlated with MD in PT (Pearson correlation=0.440, p=0.046, n=21). Verbal IQ (Spearman's rho=-0.531, p=0.013, n=21)

and FS IQ (Spearman's rho=-0.509, p=0.018, n=21) correlated with asymmetry in MD in Fus.

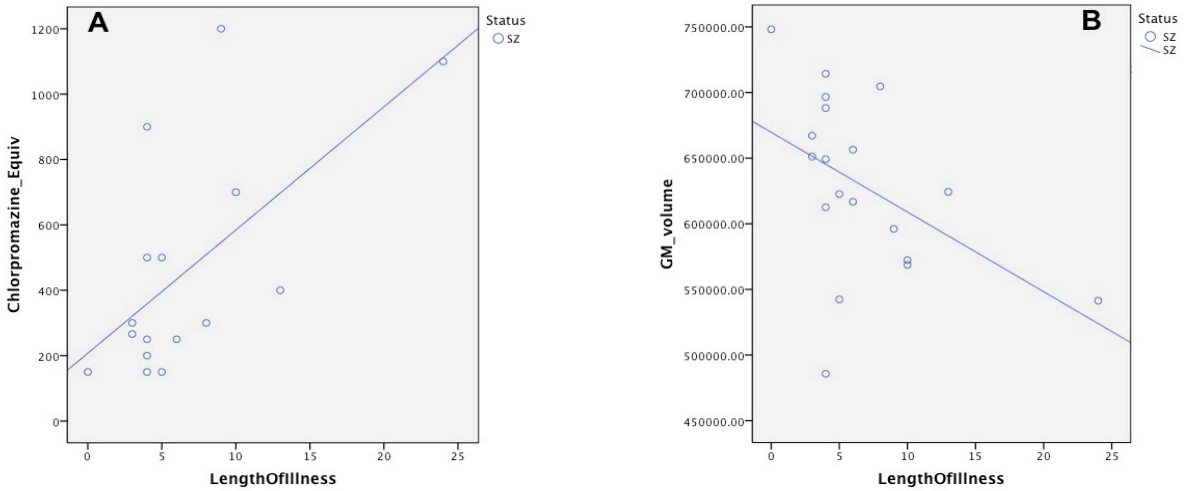
In patients, verbal IQ (Pearson correlation=0.472, p=0.042, n=19) and FS IQ (Pearson correlation=0.557, p=0.013, n=19) correlated with asymmetry in FA in BA 40; FS IQ correlated with asymmetry in FA in PT (Pearson correlation=-0.518, p=0.023, n=19). Verbal IQ (Pearson correlation=0.517, p=0.023, n=19) and FS IQ (Pearson correlation=0.562, p=0.012, n=19) correlated with asymmetry in MD in BA 39.

Hay Total correlated with MD in BA 17 (Spearman's rho=-0.651, p=0.003, n=19) and BA 39 (Spearman's rho=-0.505, p=0.027, n=19).

Clinical Variables

Age of onset was correlated with the value for the PANSS Total (Spearman's rho=-0.499, p=0.035, n=18) and with asymmetry in FA in BA 40 (Spearman's rho=0.518, p=0.023, n=19) and Para (Spearman's rho=-0.478, p=0.039, n=19). Length of illness was correlated with medication, as measured by chlorpromazine equivalents (Spearman's rho=0.533, p=0.033, n=16), and gray matter and total volume (for both, Spearman's rho=-0.0519, p=0.023, n=19) (see Figure 10).

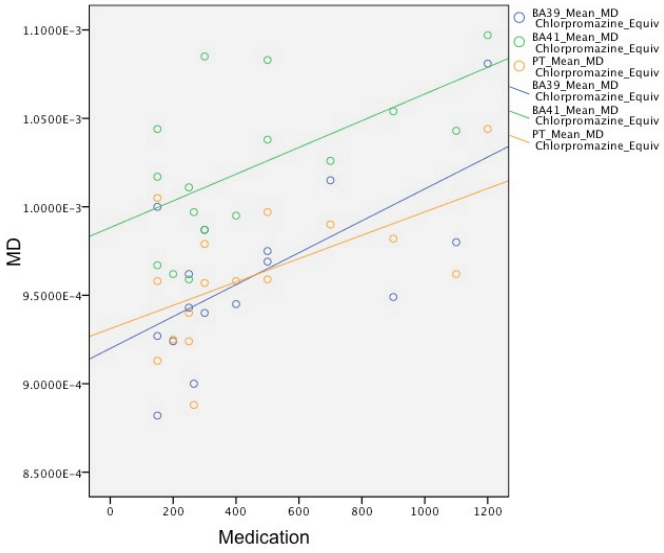
Figure 10. Relationships with length of illness



***A**: The relationship of length of illness with medication; **B**: the relationship of length of illness with gray matter volume.*

Medication correlated with FA asymmetry in BA 11 (Spearman's rho=-0.628, p=0.009, n=16), BA 39 (Spearman's rho=-0.529, p=0.035, n=16) and BA 41 (Spearman's rho=-0.506, p=0.046, n=16), and with MD in BA 39 (Spearman's rho=0.585, p=0.017, n=16), BA 41 (Spearman's rho=0.566, p=0.022, n=16), and PT (Spearman's rho=0.553, p=0.026, n=16) (see Figure 11).

Figure 11. Medication and MD



PANSS scores correlated with asymmetry in FA in BA 40 (PANSS Dis: Pearson correlation=-0.472, p=0.048, n=18; PANSS Total: Pearson correlation=-0.559, p=0.016, n=18) and in Para (PANSS Neg: Pearson correlation=0.499, p=0.035, n=18), as well as with MD in PCG (PANSS Pos: Pearson's correlation=-0.500, p=0.035, n=18; PANSS Neg: Pearson correlation=-0.489, p=0.039, n=18).

CHIPS: Validation of Method

FA and MD values from the manual segmentation were used to validate the CHIPS procedure. The manually-masked output value for FA and MD (referred to as FA Chips and MD Chips), as well as the intermediary value (used the manual masks created, but the automatic procedure, referred to as FA intermediary and MD intermediary), were compared to FA and MD values from the automatic segmentation (referred to as FA auto and MD auto) in order to compare the three methods.

Table 7. Validation of the CHIPS method

Method	BA 9		BA 39		BA 41	
	Controls	SZ	Controls	SZ	Controls	SZ
FA: Auto	0.106581	0.102541	0.108031	0.108276	0.120466	0.105923
FA: Intermediary	0.1188744	0.105358	0.120441	0.1135816	0.1326457	0.1163971
FA: CHIPS	0.122806	0.112901	0.122446	0.113618	0.135091	0.121036
MD: Auto	0.000873	0.000936	0.000926	0.000972	0.000952	0.001038
MD: Intermediary	0.0010001	0.0009885	0.0009017	0.000952	0.0009076	0.0009957
MD: CHIPS	0.000992	0.000984	0.000902	0.000964	0.000907	0.000986

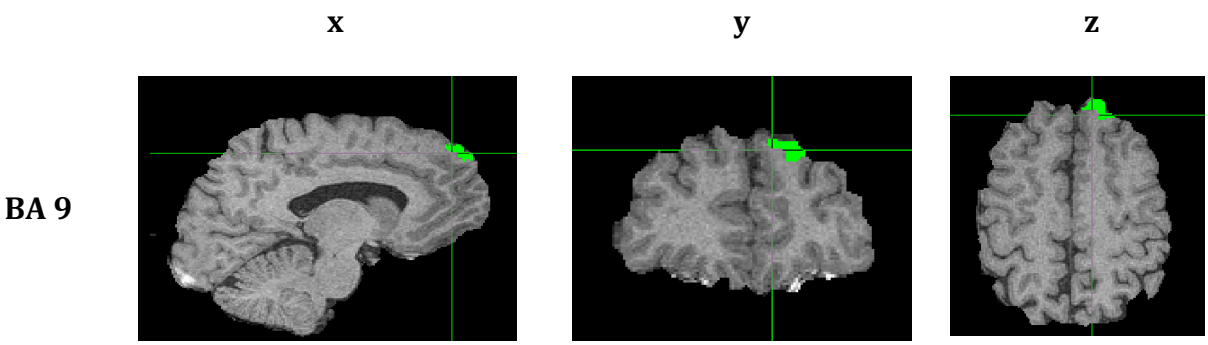
* All values are average values

A repeated-measures ANOVA was performed between methods on values of FA between diagnostic groups. Although there was no significant effect of diagnosis between subjects, there was a significant difference in FA values between the methods (F=24.057, df=1.205, p=0.000); however, these differences were not significant between regions or between diagnoses. Post-hoc tests show that that difference in FA values was

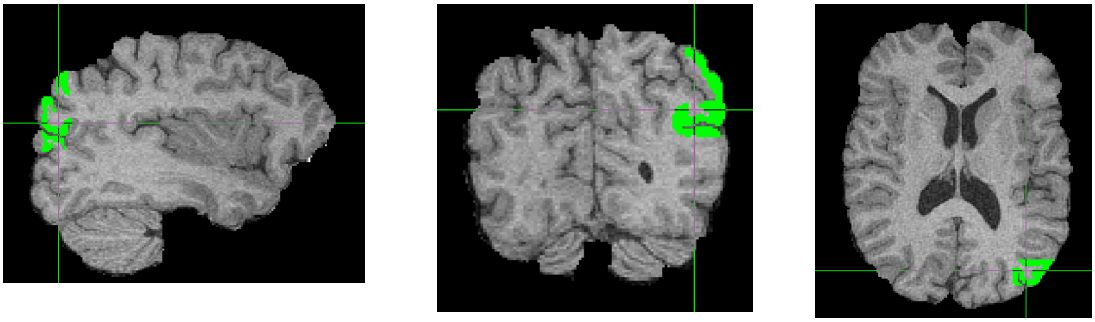
significant between the automatic method and the intermediary method (p=0.000), between the automatic method and the manual CHIPS method (p=0.000), and between the intermediary and manual CHIPS method (p=0.000).

A repeated-measures ANOVA was performed between methods on values of MD between diagnostic groups. Although there was no significant effect of diagnosis between subjects, there was a significant difference in MD values between the methods between regions (F=13.073, df=1.244, p=0.001); however, these differences were not significant between diagnoses. Post-hoc tests showed that within BA 9, the difference in MD values was significant between the automatic method and the intermediary method (p=0.009), and between the automatic method and the manual CHIPS method (p=0.011). In BA 39, the difference in MD values was significant between the automatic method and the intermediary method (t=4.031, df=19, p=0.001), between the automatic method and the manual CHIPS method (t=t=2.986, df=19, p=0.008), and between the intermediary and manual CHIPS method (t=-2.183, df=19, p=0.042). In BA 41, the difference in MD values was significant between the automatic method and the intermediary method (t=5.643, df=19, p=0.000), between the automatic method and the manual CHIPS method (t=6.906, df=19, p=0.000), and between the intermediary and manual CHIPS method (t=2.255, df=19, p=0.036).

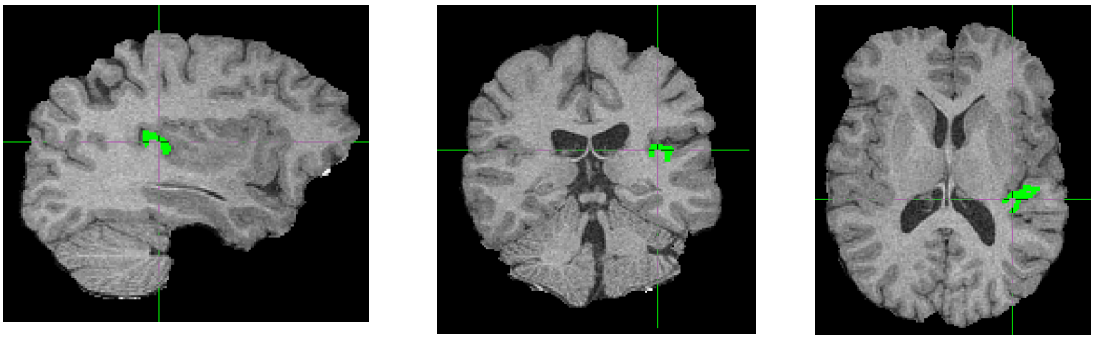
F**igure 12.** Gray matter masks of regions of interest, manual segmentation



BA 39



BA 41



CHIPS

Repeated-measures ANOVAs were performed between regions on values of cortical thickness, V1 angle, V1 dot product, scaled V1 parallel and scaled V1 perpendicular between diagnostic groups. There was no significant effect of diagnosis between subjects on any of these measures. The same was true for FA and mean regional volume in this smaller cohort. Although a repeated-measures ANOVA performed on MD values between diagnostic groups revealed that there was no significant effect of diagnosis between subjects, post-hoc tests did indicate that there was a significant difference between controls and patients in BA 39 (t=-4.835, df=18, p=0.000) and BA 41 (t=-5.456, df=18, p=0.000) on values of MD (see Figure 13).

Table 8. Results from manual segmentation (CHIPS)

Measure	BA 9		BA 39		BA 41	
	Controls	SZ	Controls	SZ	Controls	SZ
Regional Volume	2346.47	2149.89	5401.71	5278.06	1032.66	754.99
Cortical Thickness	2.22	2.15	1.83	1.88	1.34	1.38
V1 Angle	1.01	1.02	1.03	1.01	1.16	1.22
V1 Dot Product	0.50	0.48	0.48	0.49	0.38	0.33
V1 Parallel	0.00049	0.00049	0.00042	0.00046	0.00033	0.00031
V1 Perpendicular	0.00079	0.00080	0.00072	0.00075	0.00080	0.00087

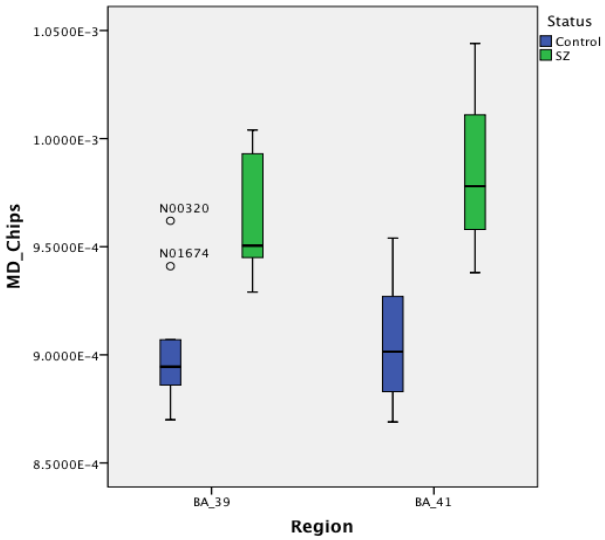
* All values are average values

Table 9. Volumetric data in CHIPS cohort

Measure	Controls	SZ
GM Volume	663707.02	641104.82
WM Volume	621740.12	631289.65
Total Volume	1327414.04	1282209.64

* All values are average values; volume is mm^3

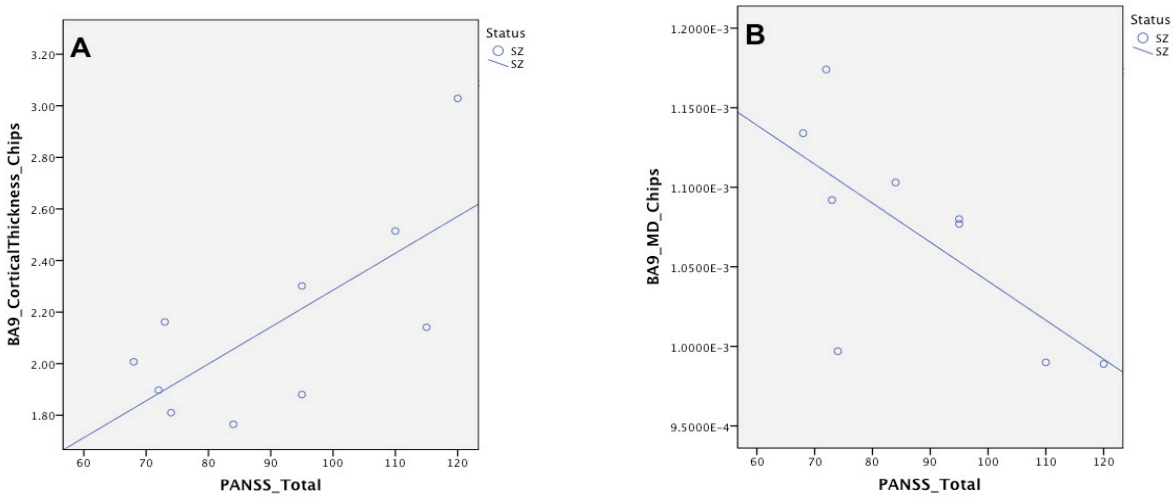
Figure 13. Differences in MD between patients and controls: CHIPS



CHIPS: Correlations

In this smaller cohort, PANSS scores correlated with cortical thickness in BA 9 (PANSS Neg: Pearson correlation=0.803, p=0.005, n=10; PANSS Total: Pearson correlation=0.714, p=0.020, n=10), as well as with MD in BA 9 (PANSS Pos: Spearman's rho=-0.767, p=0.010, n=10; PANSS Neg: Spearman's rho=-0.675, p=0.032, n=10; PANSS Dis: Spearman's rho=-0.661, p=0.038, n=10; PANSS Total: Spearman's rho=-0.875, p=0.001, n=10) (see Figure 14).

Figure 14. Relationships of Total PANSS scores in CHIPS cohort



***A**: The relationship of the Total PANSS score with cortical thickness in BA 9; **B**: the relationship of the Total PANSS score with MD in BA 9. One outlier was removed from the graph of the relationship between BA 9 MD and the PANSS score, as it visually skewed the relationship. However, the graph with the outlier included is available in Appendix A, Figure 1.*

Discussion

A novel use of an established method

This analysis describes the results of a popular and high-quality method, diffusion tensor imaging, being applied in a novel way, in the gray matter. Although some researchers have avoided measures of FA and MD in the gray matter, due to its increased isotropy, this and other studies have shown that there is valuable information to be learned from such investigations[17,18]. Although there is indeed less anisotropy in the gray matter as compared to the white matter, investigations of the gray matter in this regard can still yield data to contribute to informative analyses. In addition to the current study, this has been shown in other regions in schizophrenia. DTI has been used to investigate the gray matter in the superior temporal gyrus; no difference in FA between controls and schizophrenia patients was found[18]. This study did however show increased diffusivity in the gray matter in patients. In another study, the whole brain was analyzed for possible differences in anisotropy in gray matter using voxel-based

morphometry[17]; again, no differences in FA were found here, although there was increased MD in the parahippocampal gyrus and insula in patients.

CHIPS Validity

Before getting into a discussion of the findings of this study, it is important to report on the validity of the manual segmentation process, which was performed on a small subset of patients included in this study. The manual segmentation, called CHIPS, involves the selection of regions of interest by hand and drawing in their borders manually; this technique has the advantage of being potentially more accurate, as it draws on the knowledge of the experimenter, but is undeniably more time-consuming. The program used here to analyze the manual segmentation was called CHIPS, and this program had the added advantage of producing values for other diffusion vectors, in addition to fractional anisotropy and mean diffusivity. These values give a better description of the underlying cortical structure, especially in relationship to 'the vertical', the spatial arrangement of axon bundles and columns. The values include cortical thickness; V1 angle, the angle of diffusion from the vertical; V1 dot product, the cosine of the angle; scaled V1 parallel, the proportion of the principal diffusion direction that is parallel to the vertical; and scaled V1 perpendicular, the proportion of the principal diffusion direction that is perpendicular to the vertical. Fractional anisotropy (FA) is the degree of anisotropy of the diffusion (how directionally restricted it is), and mean diffusivity (MD) is the average diffusion value.

In validating the CHIPS method, there were three measures that were obtained: one, the value from the automatic segmentation; two, the value from the manual segmentation; and three, an intermediary value created using the region of interest from

the manual segmentation, but including all borders. In the manual segmentation, the borders were not included in the calculation of values, to avoid the partial volume effect; the intermediary value included these borders, so as to provide a value using the manual ROI but the same inclusivity of the automatic segmentation, where all borders were included in the analysis of values. All three of these values were calculated for both FA and MD.

These analyses were computed separately in controls and patients, but the results were the same in each, as shown by no significant diagnostic effects in any of the statistical tests; visual analysis of the trends confirmed this. Results indicated that the methods were significantly different from each other, for both FA and MD: in FA, the value for the automatic segmentation was lower than both the intermediary value and the manual value, and the intermediary value was between the automatic and manual values. In MD, the comparison of the methods was different in each of the three areas: in both BA 39 and BA 41, all three values differed from each other, whereas in BA 9, the automatic method differed from both the intermediary and manual methods. In MD, the trend was for the automatic segmentation to produce values that were higher than both intermediary and manual values, and then for the intermediary and manual values to average out to about the same. These results show that the manual segmentation produces higher values of FA (which indicates increased anisotropy), as well as decreased values of MD (which indicates less diffusion). These results taken together indicate that a manual segmentation method is significantly different from an automatic method, but that this difference may depend on the quality of the manual segmentation. The difference in values may for example mean that in the manual segmentation of the borders of the ROIs, voxels that were partially white matter were included in the gray matter analysis; this would account for the increased anisotropy shown in the manual

segmentation, and not the decreased diffusion. However, it has been shown that where the partial volume effect occurs between regions of white matter and gray matter, the result is decreased anisotropy[61]; this is not supported in the current model. Therefore, an alternative explanation may be that the manual segmentation process allows for more voxels of gray matter tissue to be included in the analysis, resulting in more accurate measurements. This interpretation is supported by the current study, where many of the findings from the automatic segmentation were replicated using the manual segmentation. In order to conclude that a manual segmentation should be used preferentially over an automatic segmentation, additional studies verifying the validity of the manual segmentation should be performed. Additionally, before using a manual segmentation procedure, a full review of the practicality and benefits of such a lengthy procedure must be undertaken.

Differences between patients and controls

Volumes

In an analysis of whole-brain and regional volumes, there were no differences between patients and controls. This is surprising, given that several studies have previously found differences in schizophrenia in gray matter volumes[62-64] as well as regional volumes, specifically in the superior temporal gyrus[18,65,66] and hippocampus[67,68], and generally in the frontal lobe[69,70].

However, there were changes in whole-brain volumes with age: in patients, age was negatively correlated with all three measures of whole-brain volume, implying that as they age, patients are losing brain volume. This is a common finding in the literature[71,72]. One of the popular theories that accounts for the trajectory of schizophrenia is the progressive neurodevelopmental model, which posits that

schizophrenia initially arises as an aberration in brain morphology in early development that becomes apparent at onset of psychosis[73]. Later in the illness, schizophrenia is further characterized by an altered aging trajectory that can be seen in various regions in the brain[74]; this extends even into older age[71]. This altered aging trajectory is commonly seen in reductions in gray matter[72], but changes in patients have also been observed at the microstructural level[75].

There were also correlations with these volumes; all three whole-brain volumes were positively correlated with FA values in BA 17, the primary visual cortex, in both controls and patients. The primary visual cortex is shown to be one of the first areas to mature[74,76]. This may indicate that the primary visual cortex develops before the onset of the developmental abnormalities that pervade schizophrenia[73], although it has been shown before that there are differences in volume and neuron number in BA 17 in schizophrenia[30]. Therefore the similar finding here in both patients and controls may simply indicate that as the primary visual cortex, a phylogenetically old part of the brain[74], changes, the volume of the brain itself changes.

FA and MD

In this study, there was no difference in values of FA between patients and controls. There have been differences found previously in the white matter in schizophrenia on measures of FA, primarily in the temporal lobe[5,10,13,77]. However, it has been suggested that the relevance of anisotropy may not be as applicable in gray matter as it is in white matter[18]; this is due to the more spherical shape of the FA vector in gray matter[79]. Two other studies examining FA in the gray matter also did not find any diagnostic differences[17,18].

On the other hand, mean diffusivity did differ between patients and controls; results showed that diffusivity was significantly increased in five regions in patients compared to controls: BA 9, BA 17, BA 39, BA 41, and PT. Increased diffusivity has been found before in schizophrenia, for example in the gray matter in the superior temporal gyrus[18], as well as collectively across almost all white matter structures[78]. Increased mean diffusivity in patients indicates that there are not as many barriers to diffusion in the gray matter[79] as there are in controls. Taking into account the fact that the whole-brain and regional volumes are not changing in schizophrenia, the effect found here in MD must be due to a change in the cytoarchitecture; diffusion is constrained by the structure of the cortex. Indeed, increased MD in the gray matter has been hypothesized to correlate to a decrease in neuropil, comprised of synapses and dendrites between neurons[18]. This may also however be due to a decrease in neurons or neuron density in schizophrenia; this is supported by findings in the literature of decreased neurons[30,80,81] and decreased neuron density[82-84]. However, one popular hypothesis in schizophrenia research is the reduced neuropil hypothesis, proposed by Selemon and Goldman-Rakic[85]; these authors surveyed the literature and, aided by their own work, came to the conclusion that schizophrenia was characterized by an increase in neuron density but no concurrent increase in neuron number. The hypothesis thus posits that this increase in density is due to a decrease of the neuropil, the matter comprised of synaptic connections and dendritic branches that surrounds neurons. Although there is support for this theory[86-88], results that refute it, with findings of decreased density or no change in density at all, have also been reported[22,89,90]. Therefore, if this hypothesis were to be applied to the current study, an increase in diffusivity indicates that there is a decrease of objects in the way of the diffusion. A reduction in neuropil or in neuron number or size may account for this finding[91]. As neither neuron density nor neuron number were measured in the current study it is impossible to state with certainty which the changing

factor is here; however, it is important to note that other studies that have found increased diffusivity in schizophrenia have implicated a reduction in neuropil[18,91]. Additionally, in findings from other studies of these regions, results have been reported of increased density in BA 9[92], BA 17[93], and BA 41[94], although no change has been found in density in the inferior parietal, which BA 39 is part of[89], and there are reports of decreased density in the planum temporale[95].

These results are supported by the findings from the manual segmentation procedure, completed in a smaller cohort in the current analysis. There was no difference in FA values between the diagnoses, but analyses showed that there was a significant increase in diffusivity in two out of the three regions studied, BA 39 and BA 41. Additionally, there was no difference in regional volumes between the diagnoses; this indicates, as in the larger cohort, that the changes seen in MD are not a volumetric effect and in fact are due to cytoarchitectural changes. This support of the main findings lends more credence to the hypothesis that there is increased diffusivity in schizophrenia gray matter, and that this increase is not a volumetric effect.

This finding of increased diffusivity in patients implicates altered neuroplasticity as a main effect in schizophrenia. It has been shown that there are brain changes early on in schizophrenia onset that may be the result of early alterations in development. This has been shown in groups of varying ages of onset: a recent review reported that in both childhood-onset schizophrenia and in adult-onset schizophrenia, the characteristic gray matter loss that is the hallmark of schizophrenia proceeds in a similar way in both groups, starting in posterior parietal regions and extending to frontal regions[73]. As the common factor here seems to be the onset of psychosis, it has been suggested that the brain changes seen in schizophrenia are due to underlying abnormalities in the

microstructure that are only revealed at symptom onset[96]. As patients age, this altered underlying structure is then posited to interact with normal aging processes in an abnormal way, producing the progressive losses of gray matter that have been reported in schizophrenia[97]. This altered neuroplasticity has wide implications, not only for the trajectory of brain changes, but also for cognition, disease course, and symptom severity: decreased anisotropy has been correlated with increased severity of negative symptoms[64], and decreases in volume of the frontal lobe have been linked to poorer executive functioning and greater symptoms[98]. The section on cognition will explore this further. The fact that altered neuroplasticity can be examined by studying diffusion in the gray matter, as is implied here, has implications for how future work will be conducted, as an *in vivo* study is much less invasive and has great potential for longitudinal studies.

Age

Although age did not differ between the diagnoses, relationships with age did differ between patients and controls. Importantly, the relationships with age and FA and MD that were seen in controls were generally not seen in patients; in fact, there were very few relationships with age in either of these measures in patients. This finding is significant because it implies that the changes in FA and MD that are seen in healthy controls have a biological basis: it is known that healthy aging occurs in conjunction with cortical shrinkage, which may be mediated by loss of synapses[99]. However, this may indicate that, not that there aren't structural differences with age in patients, but that the fact that patients are changing with increased age may be a function of the disease, and not the aging process; this is supported by studies in the prodrome or early-onset schizophrenia, which show that brain changes in schizophrenia are already seen at first-onset[65,100,101]. In fact, structural brain abnormalities have also been found in

those who are at risk of developing the disease (such as those with a family history), including decreases of cortical thickness[102], decreases in gray matter volume[103], and reduced FA in the white matter[9].

In controls, age was positively correlated with the total score on the Hayling test; as cognition is generally hypothesized to decrease with age[104], this finding may be spurious. However, as the age range of the control sample was 18-41, the decreases in cognitive functioning that occur with age may not have had a measurable effect on these patients, due to their younger age. Therefore, the subjects that are included here are perhaps performing better on this test as their vocabulary increases as they get older; one recent study has found that healthy controls have a longitudinal increase in IQ that is significant compared to patients[105]. This may be represented as well in the results from the current study, as controls showed an increase in semantic cognition over time, and patients showed no relationship.

There were however some important relationships with age in the patient population: as patients aged, their scores on the PANSS went down. This implies that as patients get older, they have a decrease in symptom severity. This is supported by some studies that have shown that with age, symptoms become more stable[106]. However, there is also consistent evidence that with increasing illness duration (which is often correlated with increasing age, also in this study), patients become more impaired, especially those that do not respond to treatment[107]. Additionally, this decrease in scores on the PANSS may have been a function of medication, which in this study was shown to significantly correlate with length of illness. A longer duration of illness correlated with both increased medication and increased age, and the decreased PANSS scores with age as seen here could be a function of response to medication[108].

Cognition

This analysis showed that patients and controls differed on measures of cognition: verbal and full-scale IQ were shown to be different between the diagnoses, but both were significantly higher in controls (see Figure 8). This has been shown in the literature previously[109], and is hypothesized to be one of the defining characteristics of schizophrenia[105]. It has been suggested that decreased IQ in patients correlates with poorer outcome[46,110]. As is shown in the current study, this finding may be represented structurally, as decreased scores on the Hayling test correlated with increased diffusivity in two regions in patients; an increase in diffusivity may indicate a decrease in neuron density via a loss of connections or neurons. Contrary to these results, the opposite relationship occurred with IQ and diffusivity in controls: increases in IQ were correlated with increased diffusivity. These relationships clearly do not match; however, in patients, increased diffusivity in several regions was also correlated with increased medication. Therefore, this altered relationship in patients may be due to the effect of medication; this is supported by the fact that increased diffusivity correlated with a decrease in cortical thickness in the CHIPS cohort, particularly in BA 9, as well as a decrease in symptoms. These results indicate that alterations in BA 9 in particular may predict, or at the very least correlate with, symptom severity. As various cognitive and executive functions have been attributed to BA 9, including attentional and working memory processes[111], it is not surprising to find that this region is associated with these functional correlates. Antipsychotic medications are known to be associated with brain changes in schizophrenia, including increased ventricular volume[112] and thinner cortex[113]; these results support the supposition that medication may have an effect on the amount of diffusivity.

In controls, verbal IQ was positively correlated with all three measures of whole-brain volume; the total score on the Hayling test of sentence completion (a measure of semantic cognition) was also correlated with whole-brain gray matter and total volume. These findings are supported by literature that says that IQ and, by proxy, cognition and executive functioning, does not originate from just one area in the brain (often alleged to be the prefrontal cortex) but that these functions derive from a network of involved brain areas[111].

Asymmetry

The direction of asymmetry was, for most regions, similar in patients and controls; this was true for BA 9, BA 11, BA 17, BA 39, BA 41, PT and PCG. BA 40 and Fus exhibited a dissimilar trend in patients, between FA and MD, and Para exhibited a dissimilar trend in controls between FA and MD. Table 10 shows the asymmetry trends.

Table 10. Direction of asymmetry in FA and MD in patients and controls

Region	Controls		Patients	
	FA	MD	FA	MD
BA 9	L	L	L	L
BA 11	R	L	R	L
BA 17	L	R	L	R
BA 39	L	L	L	L
BA 40	L	L	R	L
BA 41	R	R	R	R
Fusiform gyrus	R	R	R	L
Parahippocampal gyrus	R	L	L	L
Planum temporale	R	R	R	R
Precentral gyrus	R	R	R	R

* R=rightward asymmetry (right hemisphere greater than left hemisphere); L=leftward asymmetry (left hemisphere greater than right hemisphere) (R and L have been highlighted in different colors for differentiation purposes)
* Dark highlighted cells indicate where the asymmetry trend is the same for FA and MD, in both controls and patients
* Light highlighted cells indicate where the asymmetry trend is opposite for FA and MD, but the same opposition is seen in both controls and patients

Inferior parietal lobe

Both verbal and full-scale IQ were positively correlated with asymmetry in MD in BA 39 in patients, indicating that a rightward asymmetry in this region correlated with

increased IQ; verbal and full-scale IQ were shown to be positively correlated with asymmetry in FA in BA 40 in patients, indicating that greater IQ correlates with a more rightward asymmetry. Therefore, IQ in both BA 39 and BA 40 was consistent with a rightward asymmetry; although the trend in BA 40 was for a rightward asymmetry, the trend in BA 39 was for a leftward asymmetry. As has been reported, patients had significantly decreased IQ from controls. As the asymmetry in BA 40 trended positively with increased IQ, this suggests a role for BA 39 but not BA 40 in the processes of cognition. This is supported by multiple evidences of the role of BA 39 in networks related to social cognition, attention, and other executive functions[114]. This is significant, as most investigators group these two regions together as the inferior parietal lobe. However, this noncontiguity of asymmetry shows that there are differences between the regions; as the asymmetry is in FA in BA 39, this difference may take the form of a change in the amount of similarly-ordered myelin fibers that are present. Other microstructural alterations in regions of the inferior parietal lobe will be further investigated in chapter three.

Other regions

In the fusiform gyrus, controls show a rightward symmetry in FA and MD, as do patients in FA; however, patients show a leftward asymmetry in MD. This implies that in patients, there is a greater amount of diffusion in the left hemisphere than in the right; this is in contrast to controls, who show greater diffusion in the right hemisphere than the left. The literature has shown that controls tend to have a leftward asymmetry and patients to have a rightward asymmetry[42]; this is in direct opposition to the findings from the current study. Further results in the present study showed that both verbal and full-scale IQ correlated with a leftward asymmetry in MD in the fusiform in controls. As there was no relationship with cognition in patients, this may indicate that

morphological differences in the fusiform contribute to changes in cognition; this is supported by findings of altered microanatomy in the fusiform in schizophrenia[40]. An altered trend with IQ and asymmetry in the planum temporale also resulted from the current study, indicating that altered brain morphology in the planum temporale may play a role in reduced cognition. Alterations have previously been found in the planum temporale in terms of asymmetry of cortical layers and volume[36,38], indicating that changes in this region are not uncommon and may contribute to differences in relationships with IQ.

Clinical variables

The correlations with medication indicate that increased medication is correlated with more leftward asymmetries in FA in BA 11, BA 39 and BA 41. A later age of onset was correlated with rightward asymmetries in FA in BA 40 and Para. A higher total PANSS score was correlated with a leftward asymmetry in FA in BA 40. Therefore it would seem that more severe symptomology correlates with a leftward asymmetry, while those who potentially have a less severe course more generally have a rightward asymmetry. The finding of a leftward asymmetry in ventricular volume in a previous study[60] may support this.

Clinical variables: age of onset, illness duration, medication

In this analysis, both age of onset and length of illness were positively correlated with age: the older the patients were, the later the onset and the longer the duration of disease. Those who have an earlier age of onset tend to have a more severe course of disease, with greater severity of symptoms[115]. In the current study, a longer length of illness correlated with both increased age and more medication, as was discussed in the section on age. These relationships will be further explored in chapter three.

In the main cohort, age of onset was negatively correlated with the total value from the PANSS, a clinical scale used to examine symptom presentation and severity[116]. This indicates that a later onset correlates with a smaller score on the PANSS; as a greater score indicates increased impairment, this supports previous findings of lesser impairment with a later onset[115]. As there was no correlation with any of the subsets of the PANSS (positive, negative or disorganized), this implies that age of onset does not correlate with specific symptom domains, but only with general symptom severity and impairment[115].

Length of illness was negatively correlated with whole-brain gray matter and total volumes: the longer patients were ill, the greater the decrease in gray matter. This is supported in the literature[50]; however, a decrease in white matter with longer duration of illness has also been observed before[51], and this was not found in the current study, even though a decrease in white matter volume was found to correlate with increased age. Another study found similar relationships: decreased gray matter volume was found with no change in gray matter anisotropy, and decreased anisotropy in the white matter was associated with no change in white matter volume[117]. This may reflect the changes in gray matter with age that are seen here in patients, although in the current study there was no diagnostic effect on volumetric differences. As discussed previously, changes with age in the white matter are often reported in schizophrenia using other methodologies, and so this finding may be due to the use of DTI as a methodology, as suggested by Lim et al[117]. Additionally, in the current study, the value of the total brain volume tracked more closely (almost exactly, in fact) with that of gray matter, and was not as closely correlated with white matter.

Conclusions

This study provided data to examine many variables relating to brain changes in schizophrenia: the effect of clinical variables such as age of onset and medication were investigated, as were correlations with measures of cognition, as well as regional differences. A highlight of this study is that it is a novel application of a common and popular methodology, diffusion tensor imaging. The use of this method in the gray matter has not commonly been reported, as many investigators have stated that values obtained from such a method (anisotropy and diffusion) were not practical or interesting if applied to the gray matter. However, in this investigation, we have shown otherwise; not only were there relevant and significant findings, but the data generated touches on many wide-ranging issues in schizophrenia research. Increased diffusivity in patients provides support for the theory of altered neuroplasticity in schizophrenia, and the differences in age relationships between controls and patients further characterize the altered aging that occurs in schizophrenia.

It was particularly noticeable that BA 39 and BA 40, which together are generally known as the inferior parietal lobe, did not necessarily exhibit the same morphology, as shown by correlating with different measures. They showed different trends with MD and asymmetry, as well as different relationships with clinical variables such as medication and semantic tests. This is important as it supports the rationale for the methodology in chapter three of this work, where the inferior parietal lobe will be broken down into several constituent parts.

However, there are some caveats to be made regarding the collection and processing of data that are central to any *in vivo* investigation. The first is that DTI

allows for an *in vivo*, noninvasive investigation of multiple regions of interest (ROI) at the same time. These are clear advantages of DTI; however, when it comes to analysis, the issue of the partial volume effect must be accounted for. The partial volume effect is where the border between white matter and gray matter, or gray matter and cerebrospinal fluid, lies within one voxel[61]; diffusion values from these voxels in particular may therefore be unreliable. In the current study, we used a threshold of 100% to only choose voxels that were 100% gray matter, in order to prevent the partial volume effect. This may mean that the volumes from which the automatic segmentation acquired diffusion values were perhaps smaller than usual, although we believed it was important to take this measure in order to be certain that the values we obtained were accurate. In the manual segmentation, particular attention was paid to these borders when selecting the regions of interest; having the ability to select the borders by hand was a primary motivation for completing the manual segmentation. Every attempt was made to include the full volume of the structure, including its borders, but our main goal was to err on the side of caution, to ensure that the values that were obtained were accurately gray matter; in this way, there may be some voxels missing on the borders of the structures, which may have been appropriate to include in the analysis.

A second caveat to be made in regards to DTI is that even though it is a widely used tool, its histopathological correlates are uncertain. Although in the white matter, most studies interpret values for anisotropy and diffusion as being indicative of the directionality and integrity of myelinated white matter fiber tracts, a study has shown that myelin is not required to produce an anisotropic effect[118]. Other factors such as axon width and components of the extracellular space may affect the amount of diffusion, and, in particular, the direction as measured by anisotropy[61].

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Appendix A

Chapter One: Anisotropy and diffusivity in ten regions of the cerebral cortex

Table 1. Values that failed a Shapiro-Wilk test of normality: main cohort (automatic segmentation)

Region	Mean/ Asymmetry	Measure	Diagnosis	S-W Normality		
				Statistic	df	p-value
BA 11	Mean	FA	Control	0.889	27	0.007
BA 17	Mean	FA	Control	0.726	27	0.000
BA 17	Mean	FA	SZ	0.880	27	0.005
BA 39	Mean	FA	SZ	0.812	27	0.000
BA 40	Mean	FA	SZ	0.826	27	0.000
Fus	Mean	FA	Control	0.538	27	0.000
Para	Mean	FA	Control	0.670	27	0.000
BA 9	Mean	MD	Control	0.902	27	0.015
BA 9	Mean	MD	SZ	0.812	27	0.000
BA 11	Mean	MD	Control	0.862	27	0.002
Fus	Mean	MD	Control	0.606	27	0.000
Para	Mean	MD	Control	0.889	27	0.008
PCG	Mean	MD	Control	0.921	27	0.042
BA 9	Asymmetry	FA	Control	0.913	27	0.026
BA 9	Asymmetry	FA	SZ	0.855	27	0.001
Fus	Asymmetry	FA	Control	0.833	27	0.001
Fus	Asymmetry	FA	SZ	0.887	27	0.007
BA 9	Asymmetry	MD	Control	0.825	27	0.000
BA 9	Asymmetry	MD	SZ	0.820	27	0.000
Fus	Asymmetry	MD	Control	0.904	27	0.016
Fus	Asymmetry	MD	SZ	0.641	27	0.000
Para	Asymmetry	MD	Control	0.835	27	0.001
Para	Asymmetry	MD	SZ	0.878	27	0.004
BA 9	Mean	Regional Volume	Control	0.887	27	0.007
Hay Total		Clinical	SZ	0.862	19	0.010
Age of Onset		Clinical	SZ	0.833	15	0.010
Length of Illness		Clinical	SZ	0.779	15	0.002
Chlorpromazine Equivalents		Clinical	SZ	0.835	15	0.011

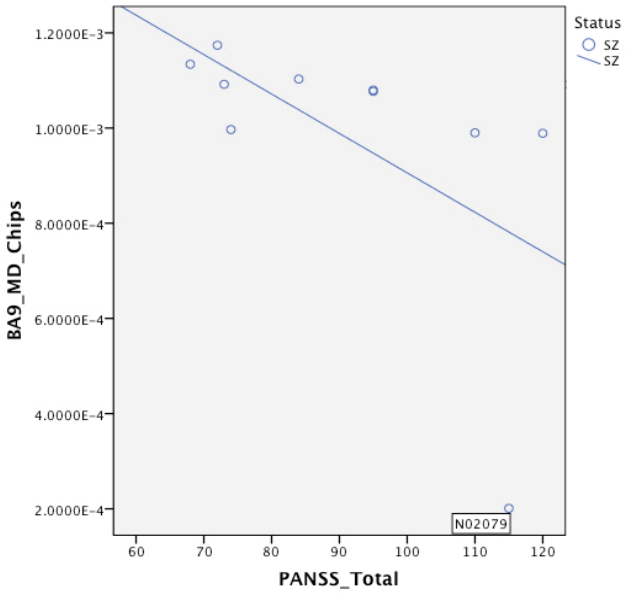
* Abbreviations: BA=Brodmann area; Fus=fusiform gyrus; Para=parahippocampal gyrus; PCG=precentral gyrus; FA=fractional anisotropy; MD=mean diffusivity; SZ=schizophrenia patients; S-W Normality=Shapiro-Wilk Test of Normality; df=degrees of freedom; p-value=significance value

Table 2. Values that failed a Shapiro-Wilk test of normality: CHIPS cohort (manual segmentation)

Region	Measure	Diagnosis	S-W Normality Values		
			Statistic	df	p-value
BA 39	Cortical Thickness	SZ	0.647	10	0.000
BA 41	Cortical Thickness	SZ	0.804	10	0.016
BA 39	V1 Angle	SZ	0.848	10	0.048
BA 9	MD	SZ	0.576	10	0.000
BA 39	Regional Volume	SZ	0.644	10	0.000
BA 41	Regional Volume	SZ	0.813	10	0.021
Hay Total	Clinical	SZ	0.841	10	0.045
Illness Length	Clinical	SZ	0.642	8	0.000
BA 9	FA Auto	SZ	0.813	10	0.021
BA 9	MD Auto	Control	0.835	10	0.038
BA 9	MD Auto	SZ	0.576	10	0.000
BA 9	MD Intermediary	SZ	0.581	10	0.000

* Abbreviations: BA=Brodmann area; Hay Total=total score on the Hayling Sentence Completion Task; MD=mean diffusivity; FA=fractional anisotropy; FA Auto=FA values from the automated segmentation; MD Auto=MD values from the automatic segmentation; MD Intermediary=MD values from the intermediary procedure of obtaining diffusivity, see text; SZ=schizophrenia patients; S-W Normality=Shapiro-Wilk Test of Normality; df=degrees of freedom; p-value=significance value

Figure 1. CHIPS: Relationship of MD in BA 9 with the Total PANSS Score (outlier included)



The inclusion of subject N02079 visually skews the relationship between MD in BA 9 and the Total PANSS Score.

Chapter Two: A meta-analysis of neuron density

Introduction

As mentioned in the general introduction, microanatomical studies in schizophrenia are numerous, and yet the findings remain inconsistent. One of the main controversies appears to be whether there is a change in neuron number or neuron density, and if this occurs in conjunction with a change in neuropil. The influential reduced neuropil hypothesis, posited by Selemon and Goldman-Rakic[1], has suggested that there is an increase in density in schizophrenia, which is due to alterations in neuropil and not changes in neuron number. Subsequently, several studies have replicated this finding in the prefrontal cortex[2] and the parahippocampal gyrus[3], among others, but decreases in density have been found in several areas, including the planum temporale[4], the hippocampus[5], and, contradictorily, in the parahippocampal gyrus[6]. Other studies have reported no change in density in the frontal cortex[7,8], or in the inferior parietal lobe[9]. It is clear that not only are there inconsistent findings, but that some findings are directly contradictory; the present study was conducted in order to clarify this issue.

The recent literature was analyzed by performing a meta-analysis on neuronal density in schizophrenia; potential relationships between neuron density and age were examined. In light of the mounting evidence for schizophrenia as a progressive neurodevelopmental disease[10-12] (for a detailed discussion, see the main introduction), age relationships were a focus. Looking at relationships with age is important in histology studies, especially as there are so few studies that have done so. Possible relationships with other clinical variables, including gender, age of onset and illness

duration, were also examined. Unfortunately, there was not enough medication information available between all the studies to justify including it in the model. However, information related to type of cell measured was extracted from each study; this was grouped as either inhibitory or excitatory. This was done due to *a priori* hypotheses of involvement of inhibitory neurons in schizophrenia: inhibitory interneurons, which moderate signaling processes between neurons and regions, have been reported to be different in schizophrenia[13]. This includes increases in density of parvalbumin- and somatostatin-immunoreactive neurons in the parahippocampus and hippocampus[5,6], as well as a decrease in density of calbindin-immunoreactive neurons in the planum temporale[4]. Disruptions in the molecular building blocks of interneurons have also been reported[14-16]. However, these findings are not consistent between brain regions or between different classes of inhibitory interneurons[17]. The present meta-analysis was able to examine findings on inhibitory neurons across studies, in conjunction with the findings on neuron density. Finally, other between-study variables such as brain collection location and method of measurement (two-dimensional or three-dimensional) were included in the analysis, and contribute to a new perspective on neuropathology research and brain collections.

Methods

An online literature search was performed to look for studies for inclusion in the meta-analysis. The online search engine 'Pubmed' was used to perform the search; two previous reviews on cell density in schizophrenia (Harrison, 1999[18], and Esiri and Crow, 2008[19]) were also examined for relevant publications. The search string used to search Pubmed for qualifying papers included "cell density" and "neuron density", "schizophrenia" in the title or abstract, and "cell density schizophrenia" in the body of the paper; an initial search was conducted in December 2009, and a final search of the

literature was done in January 2012, to include recent papers. Reference lists were also examined for relevant qualifying papers.

Inclusion Criteria

Inclusion in the meta-analysis was dependent on the following criteria: (i) the paper was written in English and available in full-form online; (ii) mean measurements of density were reported for both patients and controls, from human postmortem tissue; (iii) measurements were for neurons and not glia; and (iv) measurements were taken from the cortex and not white matter. Initially, 56 papers were identified based on the search string and reading of the abstracts; after further analysis, including a reading of the entire article, 29 papers were selected for inclusion in the meta-analysis. In the second search, 13 new studies were identified, based on the title and abstract; a more thorough reading of these studies produced 5 that were suitable for inclusion in the meta-analysis. This made for a total of 34 studies that were submitted for inclusion in this meta-analysis.

Exclusion Criteria

Papers were excluded if they did not report mean measures of density, if the study did not report values for neurons, if the measurements were not made in the cortex, or if the study was not available in English or online. Studies that measured the density of dendritic spines, molecular markers, gray-level intensity, mean spacing, or percent relative density were also excluded.

Data Extraction

In most studies, several measures of neuron density were reported, stratified by cortical layer or brain region. In all cases where it was available, the value reported for cortical layer III was selected; values reported for regions in the prefrontal cortex were also preferentially selected. A detailed description of which value was extracted from each study is available in Appendix B.

Every effort was made to obtain complete demographic details for each subject, including gender and age information, and age of onset and illness duration, if applicable. If necessary, attempts were made to contact the study authors in each instance; eleven authors replied, and six provided additional data. Data from four other studies were obtained from the website of the Stanley Medical Research Institute (studies that use tissue from the Stanley Medical Research Institute are required to post their data values on the institute's password-protected website, in order to increase collaborative efforts). There was regrettably not enough information available to analyze medication effects. A full list of demographic details is available in Table 1. Other details that were extracted from each study included the brain region of measurement; this was divided into 'frontal' regions, and 'all other' regions. Studies were only included in the 'frontal' group if they had measured Layer III; this was because there was a trend for most studies to select Layer III in the frontal cortex to study density, and this may have affected the results. The type of measurement was extracted, meaning whether the method the authors used was a two-dimensional cell counting method or a three-dimensional cell counting method. As well, the location of the brain bank used in each study was noted, as was the type of cell that was measured. These details are included in Table 2.

Table 1. Demographic details for studies selected for inclusion

Study	Author(s)	Year Published	n		Age		Gender (M:F)		Density	
			Con	SZ	Con	SZ	Con	SZ	Con	SZ
1	Benes et al[20]	1986	9	7	66.0	59.6	NA	NA	36800	26800
2	Pakkenberg et al[21]	1993	16	8	59.7	60.3	16:0	8:0	38200	43200
3	Akbarian et al[22]	1995	10	10	64.2	63.4	7:3	7:3	109.86	108.79
4	Daviss & Lewis[23]	1995	5	5	53.4	52.0	4:1	4:1	73.7	123.5
5	Selemon et al[24]	1995	19	16	43.7	40.1	10:9	12:4	1.88E+10	5.57E+10
6	Beasley & Reynolds[25]	1997	22	18	62.7	69.5	NA	NA	17.98	11.48
7	Kalus et al[26]	1997	5	5	66.0	60.2	3:2	3:2	1342	1434
8	Woo et al[27]	1997	10	10	53.9	53.6	10:5	10:5	60.3	61.2
9	Selemon et al[28]	1998	10	9	47.5	44.4	7:3	6:3	51627	62714
10	Volk et al[29]	2000	10	10	46.6	45.2	7:3	7:3	167.38	126.20
11	Thune et al[30]	2001	10	8	67	67.4	6:4	4:4	50200	47500
12	Beasley et al[7]	2002	15	15	48.1	44.2	9:6	9:6	32.85	28.11
13	Chana et al[31]	2003	15	15	48.1	44.5	9:6	9:6	95.32	93.06
14	Hashimoto et al[32]	2003	15	15	43.3	43	12:3	12:3	24.1	20.8
15	Law & Harrison[33]	2003	15	15	46.6	44.5	9:6	9:6	86.32	98.80
16	Pierri et al[34]	2003	13	13	53.1	53.2	8:5	8:5	17468	17232
17	Selemon et al[35]	2003	9	6	50.2	56.3	6:3	3:3	46400	52090
18	Tooney & Chahl[36]	2004	6	6	43.0	44.0	5:1	5:1	41.87	45.31
19	Woo et al[37]	2004	17	17	58.1	58.4	9:8	9:8	191.67	166.67
20	Chance et al[4]	2005	12	12	65.5	67.8	5:7	7:5	57.29	46.75
21	Hidalgo et al[38]	2005	13	11	46.8	46.0	8:5	4:7	8385.23	7655.18
22	Todtenkopf et al[39]	2005	32	17	63.7	54.1	18:14	17:5	1.09	0.96
23	Cullen et al[8]	2006	10	10	60.0	60.0	6:4	6:4	40000	33000
24	Garey et al[40]	2006	8	9	63.9	74.7	3:5	5:4	1036.06	1135.33
25	Dorph-Petersen et al[41]	2007	10	10	50.8	46.4	8:2	7:3	70500	67400
26	Pantazopoulos et al	2007	16	10	64.1	62.4	11:5	3:7	3.87	3.04
27	Morris et al[42]	2008	23	23	48	47.9	17:6	17:6	67.7	52.5
28	Di Rosa et al[43]	2009	13	11	68.3	65.5	7:6	6:5	1612.73	1228.00
29	Dorph-Petersen et al[44]	2009	12	12	45.2	47.3	9:3	9:3	20700	28600
30	Brune et al[45]	2010	22	20	44.1	44.7	16:6	13:7	58.47	54.45
31	Somenarain & Jones[46]	2010	8	8	60.4	60.5	4:4	7:1	1.82E+16	2.09E+16
32	Simper et al[47]	2011	16	16	66.3	66.9	7:9	7:9	4.02E+10	3.47E+10
33	Smiley et al[48]	2011	11	9	54.0	49.5	11:0	9:0	38350	37250
34	Smiley et al[9]	2012	24	24	44.3	39.8	24:0	24:0	26690.02	26628.81

* Shaded rows=cells measured in 2D
* Age and density are mean values
* n=sample size; Con=controls; SZ=schizophrenia patients
* Age is measured in years; density is either neurons/mm^2 (shaded rows) or neurons/mm^3 (unshaded rows) (see Table 2)

Statistical Model

A multilevel regression model was fitted in the statistical package 'R', in order to find variables responsible for differences in neuron density. In order to obtain normally-distributed residuals, it was necessary to take the natural logarithm of neuron density. The effects of the following explanatory variables were initially included in the analysis: diagnosis, age, gender, length of illness, age of onset, type of cell measured, and brain region of measurement (frontal or all other); within-study and within-brain collection correlations were taken into account by using these two variables as different levels in the analysis. The majority of the studies included in the analysis (eighteen) used a three-dimensional method of cell-counting; however, sixteen studies reported measurements using a two-dimensional method of cell-counting, and therefore it was necessary to include this (labeled as 'type of measurement') as a covariate. This had the effect of acting as a multiplicative conversion factor on the two-dimensional values, so as to allow for comparison between the two methods.

Interactions between the variables were examined for significant effects. The different cell types that were measured in each study were initially identified using the cell population, if described, or the staining technique, if a specific cell population was not named. However, during the formation of the model it was decided to group this variable by classifying cells as either inhibitory or excitatory; it was included in the model in this way.

Where average values were reported for patients and controls, they were weighted according to sample size; where individual subject data was available, these were entered into the model as separate data-points, with a weight of one. This weighted model was used in the analysis to account for the differences in sample size. In

this way, there were 361 data-points included in the model; this included 326 individual data-points, and 35 average data-points.

Table 2. Additional details for studies selected for inclusion

Study	Author(s)	Year Published	Region	Method	Brain Collection	Type of Cell	Age of Onset	Illness Length
1	Benes et al	1986	Frontal	3D	1	Nissl-stained	NA	NA
2	Pakkenberg et al	1993	**Other**	3D	11	Giemsa-stained	NA	NA
3	Akbarian et al	1995	Frontal	2D	2	GAD	20.8	42.6
4	Daviss & Lewis	1995	Frontal	2D	3	Calbindin	NA	NA
5	Selemon et al	1995	Frontal	3D	1	Nissl-stained	20.7	20.8
6	Beasley & Reynolds	1997	Frontal	2D	4	Parvalbumin	NA	NA
7	Kalus et al	1997	Frontal	2D	5	Nissl-stained	NA	NA
8	Woo et al	1997	**Other**	2D	3	Parvalbumin	NA	NA
9	Selemon et al	1998	**Other**	3D	1	Nissl-stained	21.3	21
10	Volk et al	2000	Frontal	2D	3	GAD mRNA	26.4	18.8
11	Thune et al	2001	Frontal	3D	11	Giemsa-stained	NA	NA
12	Beasley et al	2002	Frontal	2D	6	Parvalbumin	23.2	21.3
13	Chana et al	2003	**Other**	2D	6	Nissl-stained	23.2	21.3
14	Hashimoto et al	2003	Frontal	2D	3	Parvalbumin mRNA	25.1	18.7
15	Law & Harrison	2003	Frontal	2D	6	SMI32	23.2	21.8
16	Pierri et al	2003	Frontal	3D	3	SMI32	NA	NA
17	Selemon et al	2003	Frontal	3D	1	Nissl-stained	25.8	30.5
18	Tooney & Chahl	2004	Frontal	2D	8	Parvalbumin	NA	NA
19	Woo et al	2004	Frontal	2D	1	GAD	NA	NA
20	Chance et al	2005	Other	2D	10	Calbindin	28.8	39.1
21	Hidalgo et al	2005	Frontal	3D	9	NF200	27.3	18.7
22	Todtenkopf et al	2005	Frontal	2D	1	NA	NA	NA
23	Cullen et al	2006	Frontal	3D	10	Nissl-stained	28	31
24	Garey et al	2006	**Other**	2D	12	Nissl-stained	NA	NA
25	Dorph-Petersen et al	2007	Other	3D	3	Nissl-stained	NA	NA
26	Pantazopoulos et al	2007	Other	3D	1	Parvalbumin	23.9	36.3
27	Morris et al	2008	Frontal	2D	3	Somatostatin	25.2	23.3
28	Di Rosa et al	2009	Other	3D	10	Nissl-stained	27.5	38.1
29	Dorph-Petersen et al	2009	Other	3D	3	Nissl-stained	NA	NA

30	Brune et al	2010	Other	3D	6	VEN	20.9	23.8
31	Somenarain & Jones	2010	Frontal	3D	1	MAP2	NA	NA
32	Simper et al	2011	Other	3D	10	Nissl-stained	32.4	34.4
33	Smiley et al	2011	Frontal	3D	13	Nissl-stained	NA	NA
34	Smiley et al	2012	Other	3D	6	Nissl-stained	19.0	20.8

* Age of onset and illness length are measured in years
* Brain collection: **1 =** Human/Harvard Brain Tissue Resource Center, McLean Hospital, Belmont, Massachusetts.; University of Zagreb, Croatia; National Institute of Mental Health, St Elizabeth's Hospital, Washington, DC
2 = Brain bank, University of California-Irvine from Orange County and Ventura County, California
3 = Allegheny County Coroner's Office, Pittsburgh, Pennsylvania/ University of Pittsburgh Medical Center
4 = Corsellis Collection, Runwell Hospital, Essex
5 = University Hospital of Wurzburg; Psychiatric State Hospital of Wiesloch; Psychiatric State Hospital of Lohr
6 = Stanley Foundation Brain Consortium
7 = Heinrich-Heine University of Duesseldorf
8 = New South Wales Tissue Resource Center
9 = Cuyahoga County Coroner's Office, Cleveland, Ohio
10 = Radcliffe Infirmary, Oxford
11 = Set Hans Psychiatric Hospital, Roskilde, Denmark
12 = Charing Cross Prospective Schizophrenia Study
13=Institute for Forensic Medicine in Skopje, Macedonia; New York State Psychiatric Hospitals; Columbia-Presbyterian Medical Center
* Abbreviations: GAD= glutamic acid decarboxylase; SMI32= non-phosphorylated neurofilament protein; NF200= phosphorylated and non-phosphorylated neurofilament protein; VEN=von Economo neurons; MAP2=microtubule-associated protein 2; NA=data not available
**Note: because of the decision to only include studies in the 'frontal' group if they had measured density in Layer III, there were five studies that were performed in the frontal lobe that were not included in the 'frontal' group, as the measurements were not of Layer III neuron density; these five studies are identified by a bolded 'Other' under the 'Region' heading

Results

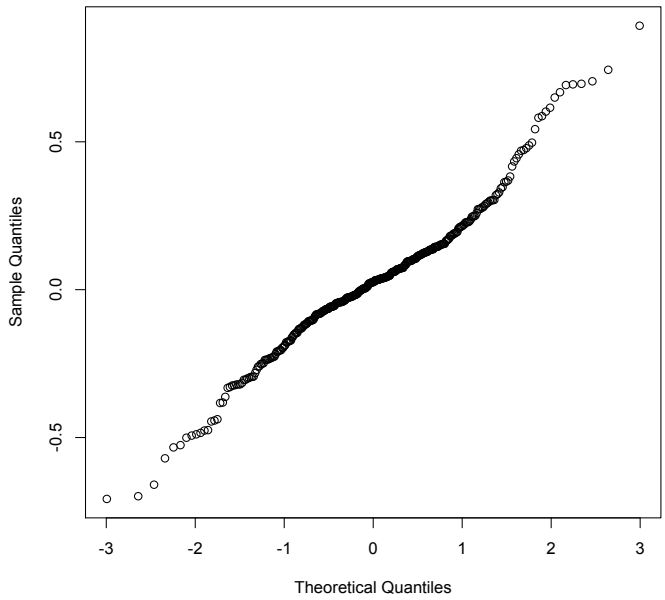
Formation of the Model

In the final analysis, a two-level model of neuron density was constructed with 'study' as the grouping variable; a three-level model, with 'study' nested within 'brain collection', resulted in no change in the quality of fit (p=0.9997). The following were included in the model as explanatory variables: diagnosis (patient/control), age, type of cell (inhibitory/excitatory), gender proportion, and duration of illness, plus interactions between age and diagnosis, type of cell and diagnosis, and gender proportion and illness duration. Type of measurement (2D/3D) was included as a covariate to adjust for the differences between two-dimensional and three-dimensional methods of cell-counting.

The significance values and model coefficients are available in Table 3. Type of cell, gender proportion and duration of illness were not themselves significant variables, but interactions with these variables contributed significantly to the model. Brain region did not significantly explain changes in neuron density, and so was not included in the model (p=0.8289).

We were assured of the goodness-of-fit of this model via an examination of the normal Q-Q plot, a graph of the residuals (see Figure 1); this confirmed that taking the natural logarithm of the neuron density values produced normality of the data. We tested for the necessity of accounting for within-study correlation as well as using a two-level model by formally testing this model against an independence model, which confirmed that a two-level model was more appropriate (p=0.0000).

Figure 1. Normal Q-Q plot of the residuals



Excluded Studies

Four studies were excluded from the final analysis. Somenarain and Jones[46] (2010) was excluded as the values obtained in this study for density were much higher than any of the other studies – on the scale of 1000000 times higher. This may have been

due to the type of cell that the density was measured in (MAP2 cells); however, it was decided to omit this study, as these values were extreme outliers that would have severely biased the model. Benes et al[20] (1986) and Beasley and Reynolds[25] (1997) were omitted from the model as they did not report gender information, and therefore they were automatically excluded. Todtenkopf et al[39] (2005) was excluded from the analysis as it was a meta-analysis and reported normalized values for density, which could not be compared to the other data. Three values had to be omitted from the model from Di Rosa et al[43] (2009), as density values were not available for those particular three subjects. Additionally, one outlier (from Hidalgo et al, 2005[38]) was removed from the analysis following examination of the residuals. This made for a total of 30 studies included in the model, with 361 data-points.

Table 3: Model coefficients and significance values

Variable	Model Coefficient	Standard deviation	Degrees of freedom	t-value	p-value
Intercept	6.937370	1.9176458	324	3.617649	0.0003
Diagnosis	0.407967	0.0911530	324	4.475624	0.0000
Age	0.006295	0.0014976	324	4.203210	0.0000
Type of cell	-3.691492	2.1674390	27	-1.703158	0.1000
Gender proportion	0.065417	0.0414283	324	1.579048	0.1153
Duration of illness	0.000387	0.0016735	324	0.231339	0.8172
Type of measurement	3.834835	2.0935252	27	1.831760	0.0780
Age : Diagnosis	-0.005606	0.0017619	324	-3.181843	0.0016
Type of cell : Diagnosis	-0.196869	0.0420224	324	-4.684863	0.0000
Gender : Illness Duration	-0.004447	0.0019185	324	-2.317906	0.0211

*Dark highlighted cells indicate significance at $p<0.01$; light highlighted cells indicate significance at $p<0.05$.

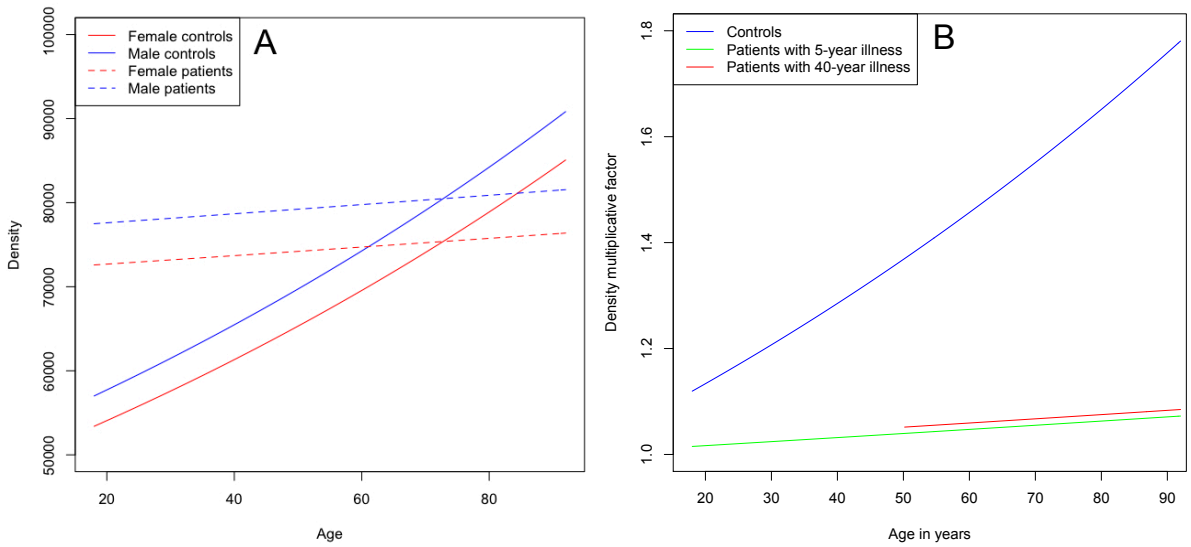
Difference between patients and controls

Neuron density was significantly different between patients and controls as a main effect ($p=0.0000$), and was higher in patients.

Interactions with age

Age had a significant effect on neuron density (p=0.0000). Additionally, the interaction between age and diagnosis had a statistically significant effect on neuron density (p=0.0016), and therefore the relationship of density with increasing age was different in patients and controls: in both groups, density increased with age. However, this effect was much smaller in patients than in controls (Figure 2A): density increased at a lower rate in patients. This effect was similar in patients with varying durations of illness (Figure 2B).

Figure 2. Relationship of density with increasing age in patients and controls



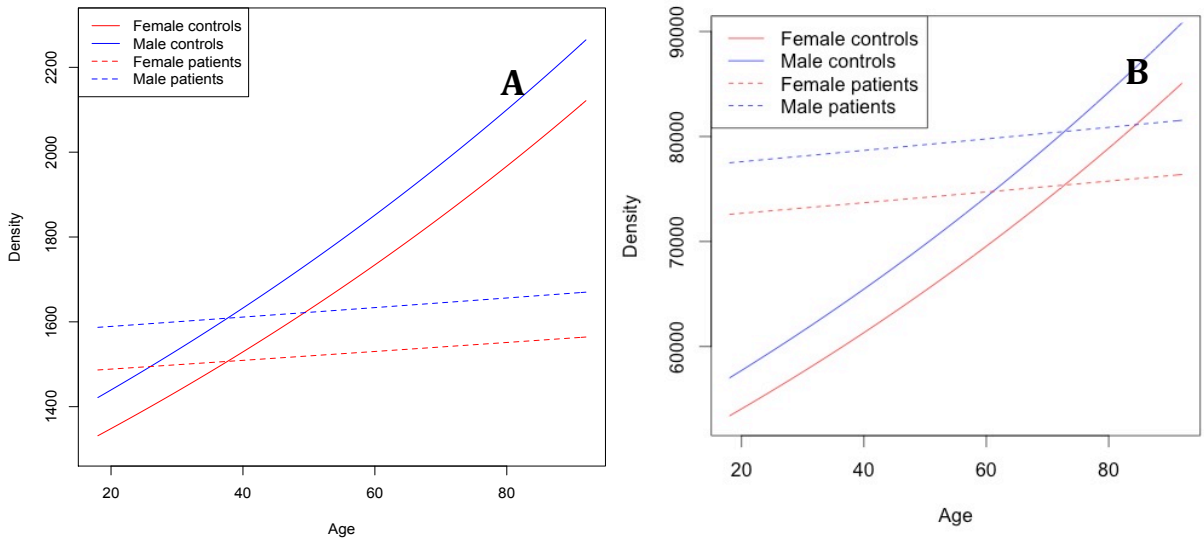
***A**: relationship of density with age; **B**: relationship of density multiplicative factor with age. Density is neurons/mm^3; age in years.*

Type of cell

There was a statistically significant interaction of the type of cell measured with diagnosis. Cells were classified as either inhibitory or excitatory; density of inhibitory cells was different in patients and controls. The following cell types were classified as inhibitory: calbindin, GAD mRNA, GAD, parvalbumin, parvalbumin mRNA, and somatostatin. In general, there was reduced density of inhibitory neurons compared to

excitatory neurons, and this effect was significantly bigger in patients than in controls (p=0.000) (Figure 3).

Figure 3. Density of inhibitory (left) and excitatory (right) neurons in patients and controls

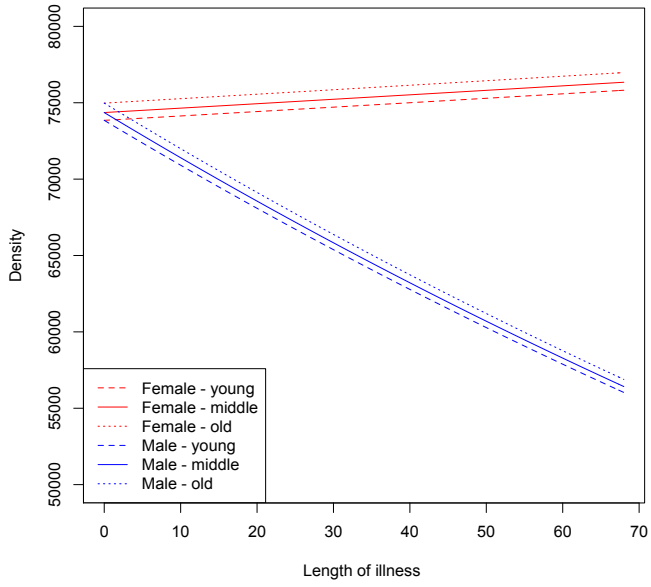


***A**: density of inhibitory neurons in patients and controls; **B**: density of excitatory neurons in patients and controls. Density is neurons/mm³; age in years.*

Gender and illness duration

There was a significant interaction between gender and illness duration in patients: in general, the longer the duration of illness, the lower the neuron density. However, this effect was significantly more pronounced in males (p=0.0211) (Figure 4).

Figure 4. Decreased neuron density with longer duration of illness in male patients



Density is neurons/mm³; age in years. The different lines indicate varying ages of onset.

Discussion

A model of neuron density

In the formation of this model, the following variables significantly contributed to the level of neuron density: diagnosis (patient/control), and age, plus interactions between age and diagnosis, type of cell (inhibitory/excitatory) and diagnosis, and gender proportion and illness duration. Overall, patients had increased density of neurons compared to controls; this finding includes data from several different brain regions, including prefrontal cortex, anterior cingulate cortex, planum temporale, fusiform gyrus, primary visual cortex, primary auditory cortex, inferior parietal lobe, and entorhinal cortex. It has been noted that findings of altered neuron density may be related to specific regions in the brain[49]; however, the benefit of a statistical model such as the one that is presented here is that we were able to test if there is, overall, a difference in neuron density in schizophrenia over the whole brain. In the formation of the model, there was no evidence that brain region had an effect on neuron density ($p=0.8289$). This indicates that overall in the brain, there is an increase in neuron density in schizophrenia; this is supported by findings of increased cell density in the parahippocampus[3], in the white matter in orbitofrontal cortex[50], prefrontal cortex[51], and parahippocampus[52], as well as increased glial density in the prefrontal cortex, mediodorsal thalamus, and anterior cingulate cortex[53,54].

One between-studies variable that did not significantly contribute to neuron density was the geographical location of the brain collections that were used in each study. Each study was coded for the location of the primary brain collection that was used to obtain samples (see Table 2); overall, there were 13 brain collections represented in this meta-analysis. We accounted for correlation within studies by

including ‘study’ as a level in a two-level model; this was justified when compared against a model with only one level (p=0.0000). Differences between brain collections may be a possible cause of the discrepancy in neuron density studies: brain collections may differ in terms of the age of the tissue, as well as the procedures and protocols used to extract and fix the tissue, although there are strict protocols in place to maintain quality[55]. Due to the fact that there are only a handful of such repositories of tissue available throughout the world, many of the brains that have been used for measurements have been included in several different studies. Because of this, we incorporated ‘brain collection’ as a separate level, but in a formal test comparing models with and without this level, there was no evidence justifying the inclusion of ‘brain collection’ as a level (p=0.9997). Additionally, once within-study correlations were taken into account, the variability between brain collections was very small (standard deviation of differences between the brain collections: 0.0009). These results support the premise that findings from studies using tissue from different brain banks can be compared. To my knowledge, there has not previously been an analysis of the effect of using different brain collections; the one presented here is an important addition to the current body of knowledge on the utilization of post-mortem tissue.

One of the issues in comparing various stereological studies is the type of measurement, ie. either two-dimensional or three-dimensional. We included type of measurement as an additive variable in the model, adjusting the logged values of neuron density to account for the difference in methods. This had the effect of acting as a multiplicative adjustment to the raw neuron density values from two-dimensional studies, in order to compare them to densities from three-dimensional studies. Sixteen studies of the thirty-four that were initially included in the analysis used two-dimensional methods of cell counting, and most of these studies occurred before 2004.

This is not to say that two-dimensional methods are not still used – one out of the eight studies that were performed in the last five years used a two-dimensional method. Three-dimensional methods have been used since at least 1986, the earliest publication date of studies included in this analysis. Three-dimensional methods are said to be more unbiased, as optical dissector techniques can be used[56]. However, a recent review concluded that two-dimensional methods may be more practical, and may even provide more accurate measurements than three-dimensional methods[56]. However, as is shown here, it is possible and desirable to account for the difference between the methods so as to analyze and make comparisons between all studies of neuron density, and not only those that use a specific methodology.

Differences between patients and controls

The reduced neuropil hypothesis states that the cause for the well-documented[57] reductions in volume in schizophrenia is a loss of neuropil, and not a loss of neurons[1]. This loss in volume has been seen in imaging studies of the whole brain[58-60] as well as individual regions, including the superior and medial temporal gyri and medial temporal lobe[61-63], Heschl's gyrus and planum temporale[64], hippocampus[65], visual cortex[41,66], medial pulvinar[67], and prefrontal cortex, precentral gyrus, and cingulate[68]. These macroscale changes should have antecedents in the microstructure, and much attention has been focused on finding the basis of this change in volume. Inconsistent findings have been the result of these investigations, with some researchers finding increases in neuron density, number, and size[2,3,50,51], some finding decreases[5,6,40,67,69], and some finding no change at all[7-9,44]. The reduced neuropil theory was proposed to account for the particular contradiction between findings of consistent neuron number but increased density in schizophrenia, and has been supported by findings particularly in

the prefrontal cortex[2,24,28,70]. However, these findings themselves are not always consistent, as is shown by findings of reduced density[5,6,40,56], no change in density[7-9], or a change in neuron number[41,49,67]. The present study provides some support for the theory of reduced neuropil, as an overall increase in density in patients was found. Although neuron density may vary regionally in schizophrenia, as has been suggested[49], this study speaks to an overall, whole-brain increase in density. Measures of neuron density are often used as proxies to make inferences about changes in neuropil, especially in conjunction with measures of neuron number. As neuron number was not included in the current study, the inferences made about a reduction in neuropil are cautious but supportive. Therefore, this model is useful in summarizing and resolving inconsistencies in the literature about changes in density in schizophrenia.

There was an interaction between age and diagnosis that significantly predicted changes in neuron density: in both groups, neuron density increased with increased age. However, this effect was much more robust in controls; this resulted in different trajectories of neuron density with age in patients versus controls (see Figure 2). These different aging trajectories provide support for the theory that there is altered aging in schizophrenia[10]. Longitudinal studies have shown that there are notable differences in aging between schizophrenia and healthy populations[71-74]; it is known from studies of healthy aging that there is a loss of synapses and dendrites, which leads to brain-wide decreases in volume[75]. This, along with other microstructural changes, has been posited to be the underlying cause behind the minor difficulties in memory retrieval and attention that occur in healthy aging[76]. These changes with age are hypothesized to occur as a result of neuroplastic processes; such processes allow for restructuring in the brain, decreasing and increasing connections as necessary, and may be instrumental in adapting compensatory recovery mechanisms in aging and dementia[77]. The brain's

ability to be plastic and to adapt to new situations is referred to as its cognitive reserve[78]. Although this is not an inherently measurable quantity, proxy measurements of cognitive reserve can be made by measuring IQ, cognition, or social functioning[79,80]. It has been shown in dementia research that those who have a greater level of cognitive reserve exhibit better cognition and less severe clinical symptoms, even when they show the same amount of brain pathology[77]. This theory has been applied to schizophrenia research. Those who have less cognitive reserve in schizophrenia, as described by IQ or educational and occupational activities[81], often show more symptoms, longer hospital admissions, and worse social functioning[79,82]. Although there are few, if any, studies directly linking cognitive reserve, proxy measures, and structural correlates, decreased anisotropy in the frontal lobe has been found to correlate with decreased IQ[6], and decreases in gray and white matter frontal lobe volumes has been correlated with a decrease in executive functioning[74].

A breakdown of the neuroplastic processes that contribute to cognitive reserve has been implicated as a potential reason for the progressive changes in age that are seen in schizophrenia[74]. The alteration in neuroplasticity is hypothesized to account for the different aging trajectory between patients and healthy controls[83]. This has been incorporated in the progressive neurodevelopmental model of schizophrenia[12]: abnormal neurodevelopment is hypothesized to cause the initial changes that lead to the development of schizophrenia. The effect of these changes is manifested at puberty, which coincides with the average age of onset[84]. Later in life, a departure from the usual neuroplastic processes causes further progressive brain changes in schizophrenia[11]. Although there is a multitude of evidence for these progressive changes, progressive worsening of symptoms or functioning has not always been found[85]. In these cases,

perhaps a greater amount of cognitive reserve is acting as a 'cushion' to prevent the effect of these additional brain changes from having an effect.

Different Cell Types

As can be expected, various different cell types were measured for neuron density between the studies; even within some studies, neuron density was measured for more than one cell population (specific details of which cell population was measured in each study are available in Table 2). In an initial analysis, there were 12 different cell categories, the most common being a Nissl stain for cell soma and dendrites. Statistical tests showed that differences between the cell types did not significantly affect neuron density. Based on an *a priori* hypothesis (that inhibitory cortical circuits are involved in pathology and resulting dysfunction in schizophrenia[13]), the studies were then split into those that had measured density of inhibitory cells and those that had measured density of excitatory cells, and this was found to have a greatly significant and different effect on patients as compared to controls. There was decreased density of inhibitory neurons compared to excitatory neurons, and this effect was larger in patients versus controls (see Figure 3). The trend for the excitatory neurons was similar to the overall trend of neuron density; both of these density and age relationships start out with patients having a higher density than controls, but then around age 80, controls overtake patients. These relationships may be similar because many of the studies that were included in the meta-analysis measured density in the frontal, or particularly the prefrontal cortex (see methods for further details), and excitatory circuits may be particularly disrupted in the prefrontal cortex[88]. This is in contrast to the aging trajectory of inhibitory neuron density, where patients still start out with a higher density, but around the age of 40, controls overtake patients. This is a

significant age, as it is also around this age that trajectories of brain volumes are hypothesized to change[71].

It has been shown that there is altered cognition in schizophrenia[13]; this especially pertains to the domains of working memory and attention, part of the executive functions that have been localized to the dorsolateral prefrontal cortex. Inhibitory cortical circuits have been shown to be abnormal in schizophrenia, particularly in the prefrontal cortex[86]. Gamma-aminobutyric acid (GABA) neurons are a class of inhibitory interneurons found in the brain[87]; they exert their influence on surrounding neurons by inhibiting them. GABA neurons have been shown to be critically important during tasks involving these executive functions[13], and it has been suggested that these neurons are decreased in schizophrenia[87]. Glutamic acid decarboxylase (GAD) is an enzyme that is primarily responsible for synthesizing GABA[88], and GAD has also been shown to be decreased in schizophrenia[22]. A reduction in GABA neurons means processes that require GABA, such as executive functioning tasks, will be impaired[86], and this is clearly seen in the disease.

With a loss of GABA neurons, the neurons that they innervate, the excitatory pyramidal neurons, are not inhibited as much[86]; that is, they experience disinhibition and therefore exhibit aberrant increased activity[89]. The results found in the current analysis support the premise that inhibitory neurons are reduced in schizophrenia; although it is true that a majority of the studies included in the current meta-analysis were performed in prefrontal cortex, neuron density was analyzed in a whole-brain fashion, and so these findings relating to decreased inhibitory neuron density in schizophrenia are applicable to the brain overall. It has been suggested that the findings

of alterations in cortical inhibitory networks in the prefrontal cortex may extend to other regions of the brain[13], and the current analysis supports this.

A recent area of inquiry in schizophrenia research is the topic of perineuronal nets; these are nets formed of chondriotin sulfate proteoglycans that surround some interneurons[89]. Perineuronal nets contribute to functions such as neuronal migration and circuit formation, and synaptic plasticity, all of which are disrupted in schizophrenia[90]; these have been shown to be disrupted in the hippocampus in an animal model of schizophrenia[89], as well as in the medial temporal lobe in postmortem brain tissue[90]. It has been suggested that this loss of perineuronal nets further reduces inhibition from interneurons, producing increased aberrant activity from pyramidal neurons[89]. To my knowledge, there are extremely few studies of perineuronal nets in schizophrenia; however, it is clear from this brief discussion that this is a topic that should be explored further, especially in the prefrontal cortex, in connection with cortical inhibitory networks.

Clinical Variables: Age of onset, duration of illness, medication

Age of onset did not significantly contribute to neuron density, and so was removed from the model. This indicates that differing ages of onset of disease do not contribute to changes in neuron density between patients and controls; this is supported by previous research that has shown that similarities between patients who have late-onset and patients who have early-onset are greater than the differences between them[91]. However, it has also been shown that patients with an earlier age of onset have greater cognitive impairment[92], and that a later age of onset is consistent with increased gray matter volume in the superior parietal lobe[93].

Duration of illness was a significant predictor of neuron density, as evidenced via an interaction with gender. Neuron density was decreased in general with a longer duration of illness, and this was significant in males. Gender effects in schizophrenia have long been a topic of continued research, and findings have shown that there are notable differences between males and females on several variables that influence or are affected by the illness, such as cognitive functioning[94], severity of illness[95], type of symptoms[96], remission[96], social functioning[97], and substance use[98]. This relative decrease in neuron density in males may be an underlying cause of some of these differences; this implies that perhaps in males, chronicity of the disease contributes to pathology in a way that it may not in females.

Unfortunately, there was not enough data available on medication from the studies included in this meta-analysis to justify including medication as an additional variable. Studies in the literature examining medication effects have often looked at differences between typical and atypical antipsychotics; for example, a comparison of haloperidol and olanzapine showed that haloperidol, a first-generation antipsychotic, was correlated with a decrease in whole-brain gray matter volume and olanzapine, a second-generation antipsychotic, was not[99]. Another study found that both typical and atypical antipsychotics affected brain morphology, but preferentially affected different structures: typical antipsychotics (first-generation) largely affected the basal ganglia and regions in the frontal and temporal cortex, while atypical antipsychotics (second-generation) often affected the thalami[100]. Notably, both of these studies were completed in first-episode patients, who had had a short medication history. Medication effects are notoriously difficult to study in neuropsychiatric research, as it is rare to find a group of individuals that are medication-naïve, and unethical to withhold treatment with antipsychotics in order to create such a group. Although there are several studies that

have been performed on individuals who were medication-naïve (or have very little exposure to medication) in the first episode of schizophrenia[99,100], longitudinal studies, which provide more information about the course of brain changes in schizophrenia without the confounding variable of medication, are less pervasive. A review of longitudinal studies concluded that antipsychotic medication was associated with decreased gray matter volume and increased ventricular volume, and that changes may be seen after only minimal medication exposure[101]; another review suggested that antipsychotics cause a marked increase in volume of the basal ganglia[102].

Studies of brain changes in schizophrenia of course must take into account the fact that medication may be having an effect on these brain changes; studies of medication effects in animal models do provide some evidence for the changes that may be due to medication, and these data are invaluable in realistically evaluating the brain changes that are found in medicated schizophrenia populations. A study looking at the effect of medication in macaque monkeys found that those who were administered antipsychotic medication had significantly smaller and lighter brains, with decreased glial cell number and increased density[103]. As the authors point out, these are often common findings in schizophrenia research, which leads to the caveat that some of the changes observed in schizophrenia may be due in part to medication, and therefore should be interpreted with caution.

Conclusions

The current meta-analysis on neuron density not only was able to provide a summary of the literature and a potential resolution to inconsistencies, it was also able to overcome many of the difficulties inherent in comparing studies that have used disparate methods. This meta-analysis of neuron density has shown that there is

increased neuron density in schizophrenia, and that there is a different trajectory of change of neuron density with age in patients and controls. These differing trajectories of the microanatomy provide support for altered aging trajectories that have been seen in volumetric analyses in schizophrenia. The overall increase in neuron density that was seen here provides support for one of the primary tenets of the reduced neuropil hypothesis, and this has implications for neuroplasticity and cognitive reserve over the course of the illness. The finding of reduced density of inhibitory neurons compared to excitatory neurons in schizophrenia lends support to the idea that there is dysfunction of inhibitory cortical circuits in the disease; taken together with a variety of evidence from the literature, this may be a putative mechanism for dysfunction in the prefrontal cortex, which contributes to the altered cognition that is a hallmark of schizophrenia. This analysis also showed that there was a reduction in density in males but not females with increased duration of illness; although this has not been reported consistently in the literature, it may imply that the underlying pathology in males and females deviates with longer illness.

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Appendix B

Chapter Two: Meta-analysis of neuron density

Table 1. Neuron density values extracted from each study

Study	Author(s)	Year Published	Region	Type of Cell	Cortical Layer
1 *	Benes et al	1986	PFC	Nissl-stained	III
2	Pakkenberg et al	1993	Frontal cortex	Giemsa-stained	NA
3	Akbarian et al	1995	DLPFC	GAD	III
4	Daviss & Lewis	1995	BA 46	Calbindin	III
5	Selemon et al	1995	BA 9	Nissl-stained	III
6 *	Beasley & Reynolds	1997	BA 10	Parvalbumin	III
7	Kalus et al	1997	ACC	Nissl-stained	III
8	Woo et al	1997	BA 9	Parvalbumin	Avg of all layers
9	Selemon et al	1998	BA 46	Nissl-stained	NA
10	Volk et al	2000	BA 9	GAD mRNA	Superficial III
11	Thune et al	2001	PFC	Giemsa-stained	All
12	Beasley et al	2002	BA 9	Parvalbumin	III
13	Chana et al	2003	ACC	Nissl-stained	III
14	Hashimoto et al	2003	PFC	Parvalbumin mRNA	All
15	Law & Harrison	2003	BA 9	SMI32	III
16	Pierri et al	2003	DLPFC	SMI32	III
17	Selemon et al	2003	BA 9	Nissl-stained	III
18	Tooney & Chahl	2004	BA 9	Parvalbumin	III
19	Woo et al	2004	ACC	GAD	Avg of II, III, V, VI
20	Chance et al	2005	PT	Calbindin	II
21 §	Hidalgo et al	2005	BA 9	NF200	III
22 *	Todtenkopf et al	2005	ACC	NA	III
23	Cullen et al	2006	PFC	Nissl-stained	III
24	Garey et al	2006	BA 11	Nissl-stained	NA
25	Dorph-Petersen et al	2007	BA 17	Nissl-stained	NA
26	Pantazopoulos et al	2007	Entorhinal cortex	Parvalbumin	All
27	Morris et al	2008	DLPFC	Somatostatin	Deep III
28 #	Di Rosa et al	2009	Fusiform gyrus	Nissl-stained	III
29	Dorph-Petersen	2009	PAC	Nissl-stained	III

	et al				
30	Brune et al	2010	ACC	VEN	Vb
31 *	Somenarain & Jones	2010	BA 9	MAP2	III
32	Simper et al	2011	PT	Nissl-stained	III
33	Smiley et al	2011	PFC	Nissl-stained	III
34	Smiley et al	2012	IPL	Nissl-stained	All

Abbreviations: PFC=prefrontal cortex; DLPFC=dorsolateral prefrontal cortex; BA=Brodmann area; ACC=anterior cingulate cortex; PT=planum temporale; PAC=primary auditory cortex; IPL=inferior parietal lobe; GAD= glutamic acid decarboxylase; SMI32= non-phosphorylated neurofilament protein; NF200= phosphorylated and non-phosphorylated neurofilament protein; VEN=von Economo neurons; MAP2=microtubule-associated protein 2; NA=data not available

* These studies were initially included in the meta-analysis but were excluded during the formation of the model, as they did not have all of the required data to support the model

§ One subject was excluded from this study; see main text

Three subjects were excluded from this study, as they did not have values given for density

Chapter Three: Minicolumn width in the inferior parietal lobule

Introduction

As discussed in the main introduction, it would seem that there are differing aging processes at work in schizophrenia; this has been posited to be due to abnormal neuroplasticity processes in aging in schizophrenia[1]. A recent unifying hypothesis, taking into account both the neurodevelopmental hypothesis of schizophrenia and the accumulated evidence of progressive changes with age, suggests that alterations during the developmental stage may only become apparent at the time of maturation[2]. These alterations then impact neuroplastic processes to create a brain morphology that is different from that seen in normal aging[1].

Minicolumns are considered to be the smallest functioning unit of modular organization in the cortex[3]. They form during development[4] and, as such, may be altered in diseases such as schizophrenia. A closer examination of exactly how minicolumns are altered may shed light on the processes of development and aging that occur in schizophrenia. Cortical layer III is optimal for minicolumnar investigations, as minicolumns can most clearly be seen in this layer; this is likely due to the high number of large pyramidal cells that are in layer III, as well as the relative thickness of layer III as compared to the other cortical layers.

Much of the neuroanatomical work that has been performed previously has focused on the frontal and temporal lobes, due to their importance in executive functioning, attention, and cognition, as well as auditory hallucinations and other

positive symptoms[5,6]. The parietal lobe has not often been investigated, even though a recent MRI study looking at disruptions in functional circuitry in schizophrenia identified the inferior parietal lobe (IPL) as a critical area involved in a network of functional disruptions, even more so than frontal regions[7]. The IPL also has several important functions that are impaired in schizophrenia, including concept of self and multisensory integration[8,9]. It has involvement in processes of social cognition[10], and has been shown to be involved in a network with Broca's area during high performance on a task of working memory[11]. Additionally, there is some evidence that the parietal lobe is involved in a system of mirror neurons[12,13]. A recent review of the literature has suggested that changes in gray matter in schizophrenia start in the parietal lobe, and spread to frontal regions[14]; this is further supported by a study that found decreased gray matter in the parietal lobe in early-onset schizophrenia[15]. Based on this evidence, and the relative lack of histological studies in the parietal lobe, the present study was performed in order to investigate differences in the microstructure in the IPL in schizophrenia.

In the current study, minicolumn width and spacing were measured in cortical layer III in schizophrenia subjects and controls in three subareas of the IPL. These measurements were examined in conjunction with relationships with age, illness duration, age of onset, and medication; as has been mentioned previously, it is important to investigate these clinical variables where possible, as there are as yet no fully consistent relationships with any of these variables and schizophrenia (see main introduction for a more complete discussion of these issues). Gender was not investigated within this study, as all subjects were male. In this sample, data on substance use, including tobacco, alcohol, and drugs was also available, as was suicide status; correlations between these variables and other demographic measures are of

interest, in order to further characterize this patient cohort. Additionally, as this was a postmortem study, tissue variables such as brain weight, postmortem interval (PMI) and brain pH were made available; these will be discussed in conjunction with results from the IPL measurements.

Of interest, there was also available with this patient cohort scores on a measure of insight; although this was not a very robust measure, relationships with other variables were investigated. Insight in this case means awareness of the schizophrenia condition[16]; increased insight has been linked to higher IQ[16], which has implications for disease progression and cognitive reserve[17].

Methods

Subjects

This study was carried out on the Parietal Collection from the Stanley Medical Research Institute (SMRI), which consists of 48 subjects (all Caucasian males), 24 of whom are schizophrenia patients and 24 of whom are matched controls. Controls were matched on age, sex, race, PMI, pH and storage time[8]. Demographic characteristics can be found in Table 1; supplementary information can be found in Table 2.

Table 1. Demographic characteristics

Subjects	Age	Brain Weight	PMI	Brain pH	Age of onset	Duration of illness	Lifetime antipsychotic
Controls (n=24)	44.29 (9.26)	1479.29 (102.92)	24.42 (10.79)	6.68 (0.21)	-	-	-
Patients (SZ) (n=24)	39.83 (10.70)	1469.42 (100.84)	29.08 (11.59)	6.53 (0.23)	19.04 (5.77)	20.79 (10.33)	79002.08 (79529.62)

*Age, age of onset and duration of illness are measured in years; brain weight is measured in grams (g); PMI is measured in hours; brain pH is measured on the pH scale (1-14); lifetime antipsychotic medication is measured in fluphenazine equivalents (mg)
* Values are mean (SD)

Table 2. Supplementary information

Subjects	Hemisphere		Drug use		Alcohol use		Suicide status		Smoking at TOD		Insight	
	Left	Right	No history	History	No history	History	No	Yes	No	Yes	Poor/Fair	Good
Controls	14	10	18	6	14	9	24	0	12	5	-	-
Patients	14	10	5	18	6	18	18	6	2	18	15	9

*TOD=time of death
*Values are number of subjects in each category

Tissue Samples & Neuroanatomy

Sections were taken from the angular and the supramarginal gyri (Brodmann Areas 39 and 40, respectively), and were formalin-fixed and cryo-protected in a 30% sucrose buffer solution[18]. They were sliced at 30μm and Nissl-stained with cresyl violet. Within both the patient subgroup and the control subgroup, 14 cases had sections taken from the left hemisphere and 10 cases had sections taken from the right hemisphere.

Measurements were made in the posterior portion of area PF, known as PFm (roughly translates to BA 40), and the anterior and posterior portions of area PG (roughly translates to BA 39), as defined by Von Economo and Koskinas[19], and Caspers et al[20]. Area PFm had been delineated previously on the section slides by a colleague (S.E.); five successive slices from each subject had been selected as being the most representative of the area, and measurements were taken from these five. The definitions postulated by Von Economo and Koskinas[19] and Caspers[20] et al were used to differentiate area PFm, as well as area PG.

The posterior portion of PF is labeled as PFm; this is denoted cytoarchitecturally by indistinct borders between cortical layers II and III, and between layers V and VI (see Figure 1A). The border between layer VI and the white matter is difficult to recognize; the low cell density in layer V in PFm is consistent in areas PGa and PGp as well. Also

consistent with PGa, and therefore difficult to distinguish from PFm, is the clearly separated Layer IV from layers III and V, and the noticeable increase in pyramidal cell size in layer III as they appear deeper through the cortex. I found that PFm was characterized best by the indistinct borders between II and III, which are in direct opposition to area PGa, as well as the location of layer IV (in PFm layer IV is in the deepest third of the cortical width, whereas in PGa it shifts to the middle). As well, once the shift into PGa has begun, the border between layers V and VI becomes much clearer, and this was also a tell-tale sign of a shift between areas.

PGa is the anterior portion of area PG, and is directly caudal to PFm; it is defined as having a prominent cortical layer IV that divides the cortex into two distinct bands, a narrow layer II, a dramatic increase in pyramidal cell size from superficial layer III to deep layer III, a distinct border between layers V and VI, and an indistinct border between layer VI and the white matter (see Figure 1B). Characteristics that were most useful to distinguish PGa were the narrow layer II, the increase in cell size in layer III, and the 'smudged' border between layer VI and the white matter. In transitioning to PGp, most noticeable was the broadening of layer II, the homogenization of pyramidal cell size in layer III, and a sharp border between layer VI and the white matter (see Figure 1C). Other identifying characteristics of PGp were an indistinct border between layers II and III and a lower cell density in layer VI than in area PGa. Posterior to area PGp was the occipital area known as OA; this was identified through the dramatic decrease in size of cells in layer V and the relative widening of layers II and III.

Figure 1 Cortical layers in PFm, PGa, and PGp

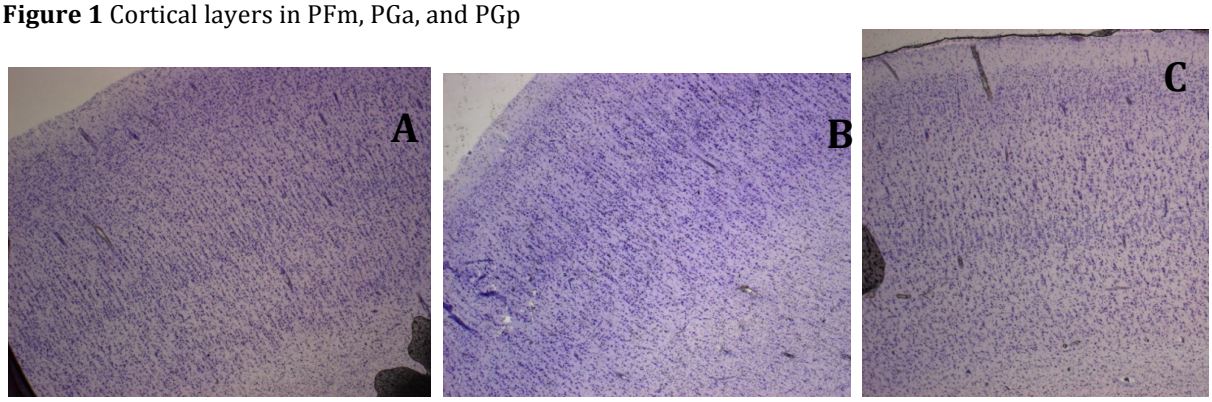


*Figure **1A** shows the cortical layers in area PFm; **1B** shows the cortical layers in area PGa; **1C** shows the cortical layers in area PGp. Particularly notice the borders between layers II and III in A and B, as well as the middle-ing of layer IV and thin layer II in B. In C, notice the thickening of layer II and indeterminate border between II and III.*

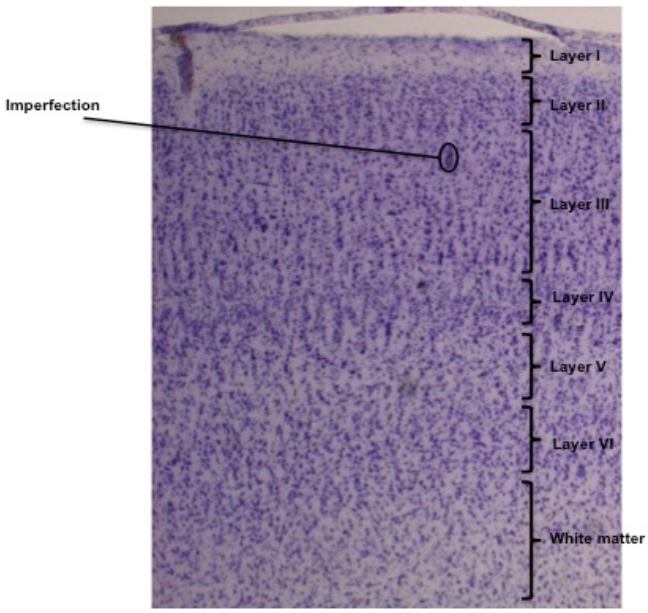
All of the slices caudal to the five identified as PFm were photographed using a microscope and camera. Images from each slice were carefully examined for the criteria listed above, and were identified as being representative of one of the above three regions. High-resolution photographs of a lateral view of the dissected hemisphere, before the sections were removed, were provided by the SMRI (see Figure 3). In most cases, the lateral fissure and superior temporal sulcus, and, in some cases, the central sulcus, had been identified and drawn on the photographs; however, in each case, these were checked for anatomical correctness. By using the definitions given by Caspers et al[20], the broad outlines of areas PGa and PGp were drawn on the photographs. These were used as a secondary, macroscale guideline to identify the borders of these areas, including the ventral and dorsal borders of PGa and PGp. These borders were subsequently transferred and mapped onto the slides (see Figure 3).

Measurements

Minicolumn width measurements were taken from layer III; minicolumns are most clearly observable in this layer, due to the high density of cells ordered in a radial fashion in comparison with the other cortical layers (see Figure 2). A minicolumn was

considered to be composed of a cell-dense core and a cell-sparse neuropil area surrounding the core. The method for measuring width of the minicolumn has been adapted from a semi-automated procedure to minimize potential human error; this procedure has been described in detail[3] and validated in Casanova and Switala[4]. Briefly, a specialized computer program has been created that is able to measure minicolumns by identifying pyramidal cells, due to contrast in shape and gray level intensity with the neuropil. A column of cells is recognized based on 'nearest-neighbor measurements', identification of clusters of cells that are radially oriented, plus the alternating neuropil space in between columns[21]. Each minicolumn was measured center-to-center, as described in Chance et al[1].

Figure 2. Minicolumns and the six-layered cortical structure

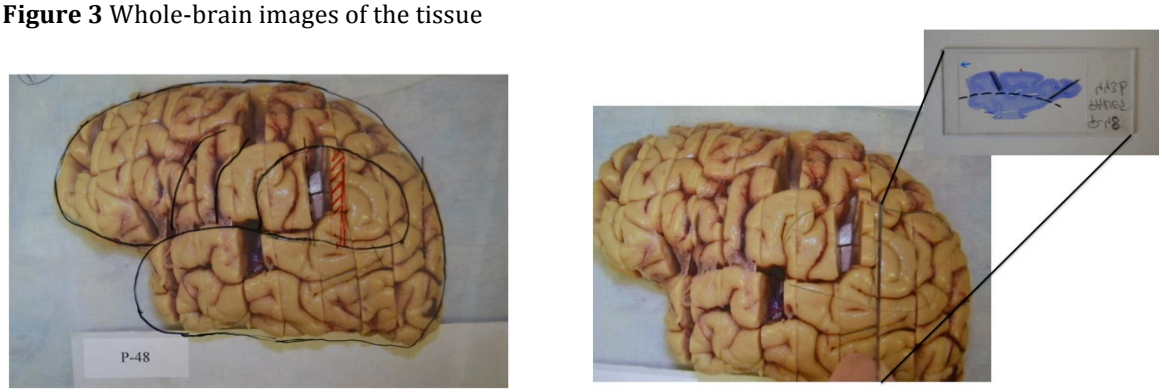


An example of the six-layered cortical structure. Layer III is optimal for minicolumn measurements, as the arrangement of cells in the vertically-oriented column can most clearly be seen in this layer. The oval indicates an example of a tissue imperfection that would be deselected during the image analysis.

Slides were imaged using an Olympus BX40 microscope under 4x magnification and an Axiocam camera attached to the microscope. Photographic images were taken of each of the slices in order to facilitate regional identification. Using these images, slides were identified as being from either area PGa or area PGp; the ventral and dorsal

borders for these areas were delineated on the slides using information from the whole-brain images provided by SMRI (see Figure 3). After areas PGa and PGp had been identified and defined in all subjects, a maximum of 10 slides per subject were randomly selected for further analysis (slides were randomized using number randomizer http://www.random.org). In all cases an attempt was made to obtain a minimum of 10 measurements per subject, from 10 different slices in PGa and PGp, and from five different slices in PFm.

Figure 3 Whole-brain images of the tissue



An example of the whole-brain images provided by the SMRI, with the general area of the IPL outlined; using the images, and knowledge of sulci and gyri, the slides were mapped onto the brain.

In area PFm, five slices per subject were identified previously by a colleague; the definitions of Caspers et al[20] were also used to divide this region. After matching up the slides with the brain as shown in Figure 3, neuroanatomical landmarks such as the lateral fissure and central sulcus were located. These were used for orientation purposes, and to choose which slides to select for sampling. In order to maintain consistency, these five slides per subject that were selected by this colleague were used to measure minicolumn width in the PFm, with two measurements being made per slide. Less than two measurements per slide were made for subject P21, with only nine measurements being made from five slides. This is due to one slide (number 222) having a particularly high number of tearing and holes in the tissue, rendering much of the tissue not optimal for minicolumn measurements. It was possible to make two

measurements from each of the other four slides for this subject, ending up with a total of nine measurements for subject P21. More than two measurements per slide were made for subjects P2, P3, P36 and P38. These subjects were analyzed first and therefore more images were taken to gain familiarity with the software, tissue, and procedures. It was decided to include these additional measurements in the final statistical analysis, in order to make full use of the available data. Full details of the total number of slides and measurements per slide in each subject are available in Appendix C. PFm data was not available for two subjects (P5 and P46), as the tissue sections that were made available did not include PFm for these subjects.

In PGa, there were 14 subjects where it was possible to take one measurement from each of 10 slides, for a total of 10 measurements per subject. For two subjects (P21 and P25) there were fewer than five slides for each subject from which measurements were possible (four slides in subject P21 and three slides in subject P25). For these two subjects, more than two measurements per slide were taken, so that there was a total number of 10 measurements per subject. A number randomizer was used to identify from which slides additional measurements should be taken. For the other 32 subjects, the number of slides per subject from which it was possible to take measurements ranged from five to nine. For these subjects, a maximum of two measurements were taken per slide, to total 10 measurements per subject. Again, the number randomizer was used to identify from which slides additional measurements should be taken. Full details of the total number of slides available for measurement and measurements per slide in each subject are available in Appendix C.

In PGp, there were 30 subjects where it was possible to take one measurement from each of 10 slides, for a total of 10 measurements per subject. For two subjects (P38

and P42) there were fewer than five slides for each subject from which measurements were possible (four slides in subject P38 and one slide in subject P42). For these two subjects, more than two measurements per slide were taken, so that there was a total number of 10 measurements per subject. A number randomizer was used to identify from which slides additional measurements should be taken. For the other 16 subjects, the number of slides per subject from which it was possible to take measurements ranged from five to nine. For these subjects, a maximum of two measurements were taken per slide, to total 10 measurements per subject. Again, the number randomizer was used to identify from which slides additional measurements should be taken. Full details of the total number of slides available for measurement and measurements per slide in each subject are available in Appendix C.

In general, area PGp was larger than area PGa, and so there were often more slides available to measure PGp than PGa. Specific numbers of slides available for each area in each subject are available in Appendix C.

Image Acquisition & Analysis

Each slice selected for analysis was examined in detail under a microscope in order to determine optimal measurement sites. An optimal measurement site visibly and identifiably had all six cortical layers, and was relatively free from staining residue and holes or tears in the tissue. Measurements were preferentially taken from straight and not highly curved parts of the cortex, as a bend in the cortex distorted the minicolumns and potentially could have given unreliable results. We also made sure to identify measurement sites that were distinctly separated from other possible imaging sites, so as not to have overlap between one image and the next. In order to ensure that

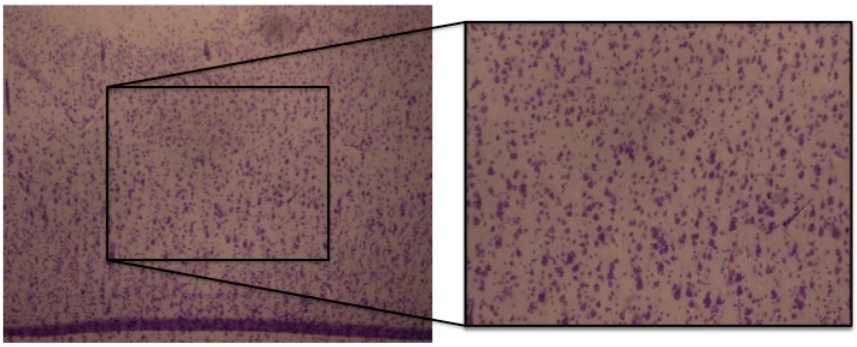
measurements were taken from the surface of the cortex, a border was drawn on each slide 10mm from the cortical surface, and all measurements were made above this line (see Figure 4). Each slice was examined for as many measurement sites as fit these criteria; these sites were then randomized (using number randomizer http://www.random.org) to identify from which sites measurements would be made. 10 measurements were made per subject. In this manner, all attempts were made to ensure that the areas from which measurements were taken were as unbiased and accurate as possible.

Figure 4 Cortical border

A section from area PGp showing the 10 mm cortical border that was used in all slices, to avoid sampling too far into the cortex. The arrow indicates the superior (top) of the brain, and the red dot indicates the location of the image taken from this subject. The two solid lines indicate the borders that denote the region of PGp in this slide.

In order to facilitate later analysis, layer III was positioned in the middle of the microscope screen, with the minicolumns as vertical as possible (accomplished by adjusting the stage of the microscope). Once each image was taken with the Axiocam, it was processed using Adobe Photoshop software. Each image was cropped to retain the central 50% of the image; this ensured that layer III was emphasized in the analysis. Each image was also resized using a standardized procedure in Photoshop, in order to fit optimal specifications for Matlab, the software used to compute minicolumn width (see Figure 5).

Figure 5. Image processing in Adobe Photoshop



The left image is the original photo of the tissue, taken using an Axiocam attached to a microscope. The right image is an enhanced image of the same photo, processed using Adobe Photoshop in order to highlight layer III.

After each image was processed in Photoshop, it was analyzed using Matlab software, where a predefined, semi-automated procedure computed minicolumn width measurements, as well as a value for spacing (the width of the neuropil 'space'). Once each image had been loaded into Matlab, there was the opportunity to "deselect" imperfections (ie. staining residue, folds or tears in the tissue, etc). Layer III was selected as the area from which to obtain width measurements. The software then computed the width of each minicolumn within layer III, and produced an average of these values for each image; this value was saved in an output file. This procedure was repeated individually for each image, resulting in a total of ten values each per PFm, PGa, and PGp, for each of the 48 subjects. The 10 values per area in each subject were then averaged, to give a mean value for each of PFm, PGa, and PGp in each subject.

Statistical Analysis

Standard statistical tests were used to analyze the data, including repeated-measures ANOVA, tests of normality, independent-samples t-tests, correlations, and, where applicable, nonparametric tests of differences and correlations.

Four continuous variables (drug use, alcohol use, smoking at time of death and insight) were redefined so that they became binomial. This was done to consolidate the data and facilitate analysis.

Results

Statistical analyses were carried out using SPSS (version 20). Minicolumn width and spacing values can be found in Table 3.

Table 3 Minicolumn width and spacing, measured in μm

Subjects	PFm		PGa		PGp	
	Minicol.	Spacing	Minicol.	Spacing	Minicol.	Spacing
Controls	27.67 (0.92)	14.37 (0.37)	27.02 (1.13)	13.98 (0.33)	27.29 (1.00)	14.00 (0.33)
Patients	27.66 (1.31)	14.36 (0.43)	27.71 (1.03)	14.18 (0.37)	27.33 (1.13)	14.03 (0.43)

* Sample size (n) is 24 for controls and 24 for patients in areas PGa and PGp for both minicolumn width and spacing; n=23 for controls and 23 for patients in area PFm for both minicolumn width and spacing.
* Minicol. = Minicolumn width
* Values are mean (SD)

Tests of Normality

The Shapiro-Wilk test of normality was carried out for the minicolumn and spacing measurements from PFm, PGa and PGp; none of these reached significance. Normality histograms were drawn for each variable in order to confirm this. Therefore, it was accepted that these data were consistent with a normal distribution. The Shapiro-Wilk test was also carried out on the demographic variables, and was significant for age in patients, age of onset, duration of illness and medication in patients; therefore, any analyses using these variables was done using nonparametric tests. Details of the failed Shapiro-Wilk tests are reported in Table 4.

Table 4 Details of variables that failed a Shapiro-Wilk test of normality

Variable	Statistic	df	Significance (p-value)
Age (SZ)	0.912	23	0.046
Age of onset (SZ)	0.912	24	0.039
Duration of illness (SZ)	0.908	24	0.032
Lifetime antipsychotic (SZ)	0.838	24	0.001

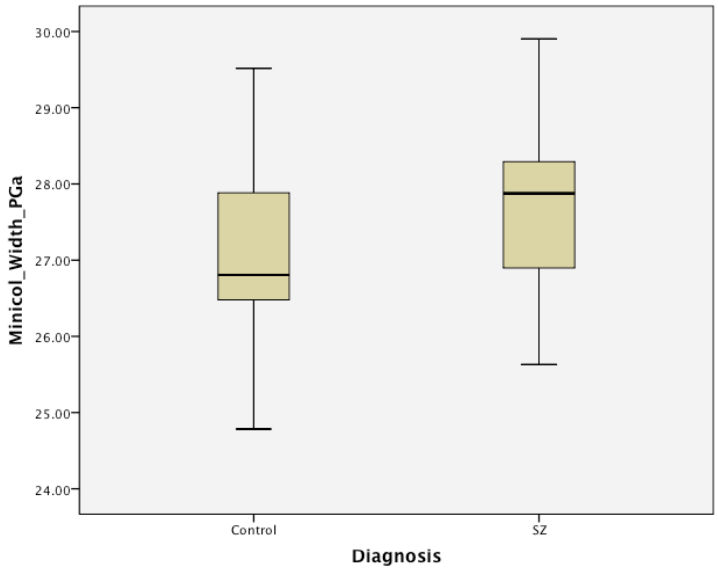
* Age, age of onset, and duration of illness measured in years; lifetime antipsychotic measured in fluphenazine equivalents (mg)

Minicolumn width

A repeated-measures ANOVA between diagnostic groups was performed on minicolumn width within the three regions. A Box's Test for equality of covariance matrices was not significant, indicating that covariance matrices were equal between the diagnoses. A Levene's Test for equality of error variances was not significant for any of the regions, indicating that covariance matrices were equal between the diagnoses in each region. There was no difference between controls and patients on a measure of minicolumn width. However, there was a significant difference in minicolumn width between areas (df=2, F=3.317, p=0.041), as well as a significant difference between areas and diagnoses (df=2, F=4.294, p=0.017). This led to additional analyses of these differences.

Post-hoc paired-samples t-tests were used to investigate the differences in minicolumn width between the areas. Within controls, areas PFm and PGa were significantly different from each other (t=2.948, df=22, p=0.007). Within patients, none of the areas were significantly different from each other. An independent-samples t-test showed that minicolumn width in PGa was significantly different between patients and controls (t=-2.190, df=49, p=0.034) (see Figure 6).

Figure 6. Minicolumn width in PGa showed a difference between patients and controls



This difference was significant at p<0.05.

Further repeated-measures ANOVAs were also performed, using other demographic and clinical measures as covariates, including age, brain weight, brain pH, PMI, hemisphere, alcohol use, drug use, and smoking at time of death. A within-subjects interaction between minicolumn width in each area and diagnosis was significant when several of these were added as covariates, including age (df=2, F=4.706, p=0.011), brain weight (df=2, F=4.072, p=0.020), drug use (df=2, F=4.488, p=0.014), and smoking at time of death (df=2, F=5.832, p=0.005). Tests of between-subjects effects were significant for age (df=1, F=6.221, p=0.017) and brain weight (df=1, F=10.590, p=0.002).

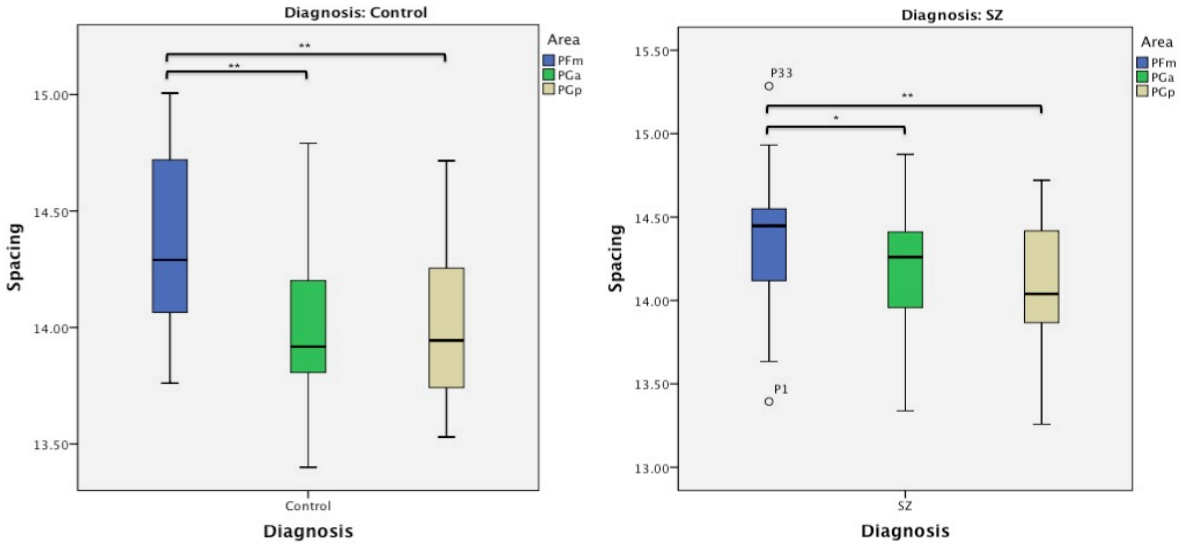
Spacing

A repeated-measures ANOVA between diagnostic groups was performed on spacing within the three regions. A Box's Test for equality of covariance matrices was not significant, indicating that covariance matrices were equal between the diagnoses. A Levene's Test for equality of error variances was not significant for any of the regions, indicating that covariance matrices were equal between the diagnoses in each region.

There was no difference between controls and patients on a measure of spacing. However, there was a significant difference in spacing between the three areas (df=2, F=21.587, p=0.000). This led to additional analyses of these differences using a paired-samples t-test.

Post-hoc paired-samples t-tests were used to investigate the difference in spacing between the areas. This was performed separately in controls and patients, in order to facilitate diagnostic comparisons. In controls, areas PFm and PGa were significantly different from each other (t=5.359, df=22, p=0.000), as were areas PFm and PGp (t=4.965, df=22, p=0.000). In patients, areas PFm and PGa were significantly different from each other (t=2.085, df=22, p=0.049), as were areas PFm and PGp (t=3.627, df=22, p=0.001). These differences are shown in Figure 7.

Figure 7. Difference in spacing across three regions in controls and patients



*Relationships with spacing in all three regions. ** indicates significance level of p<0.005; * indicates significance level of p<0.05.*

Further repeated-measures ANOVAs were also performed, using other demographic and clinical measures as covariates, including age, brain weight, brain pH, PMI, hemisphere, alcohol use, drug use, and smoking at time of death. A within-subjects test of differences between the areas was significant when several of these were added

as covariates: PMI (df=2, F=6.708, p=0.002), alcohol use (df=2, F=4.165, p=0.019), drug use (df=2, F=6.392, p=0.003), and hemisphere (df=2, F=4.925, p=0.009).

Post-hoc Analyses: Supplementary Variables

Supplementary variables, including information on drug use, alcohol use, hemisphere, suicide status, smoking at time of death and insight into the disease, were analyzed. Between the diagnoses, suicide status was significantly different (p=0.022), as was alcohol use (p=0.019), drug use (p=0.000), and smoking status at time of death (p=0.000). For these tests, a two-sided Fisher's exact test was used. There was no effect of hemisphere.

In patients, independent-samples t-tests showed that there was a difference in brain weight (t=-2.295, df=20.009, p=0.008) and age of onset (t=2.403, df=22, p=0.025) between those who did and did not have a history of drug use. Spacing in PFm was significantly different between those who had a history of alcohol use and those who did not (t=-3.151, df=21, p=0.005), as was drug use (Fisher's exact test, 2-sided: p=0.018) and smoking status at time of death (Fisher's exact test, 2-sided: p=0.032). Several variables were significantly different between suicide status, including minicolumn width in PFm (t=3.417, df=21, p=0.003), minicolumn width in PGp (t=2.813, df=22, p=0.010), medication (t=3.758, df=20.182, p=0.001), as well as age (p=0.002), minicolumn width in PGa (p=0.009), and duration of illness (p=0.000) (these last three significant on a nonparametric Mann-Whitney U test). There was also a difference in spacing in PFm between patients who had poor or fair insight into their condition and those who had good insight (t=2.203, df=21, p=0.039).

In controls, there was a significant difference in minicolumn width in PFm (t=2.091, df=21, p=0.049) and brain weight (t=2.587, df=22, p=0.017) between those who had a history of drug use and those who did not.

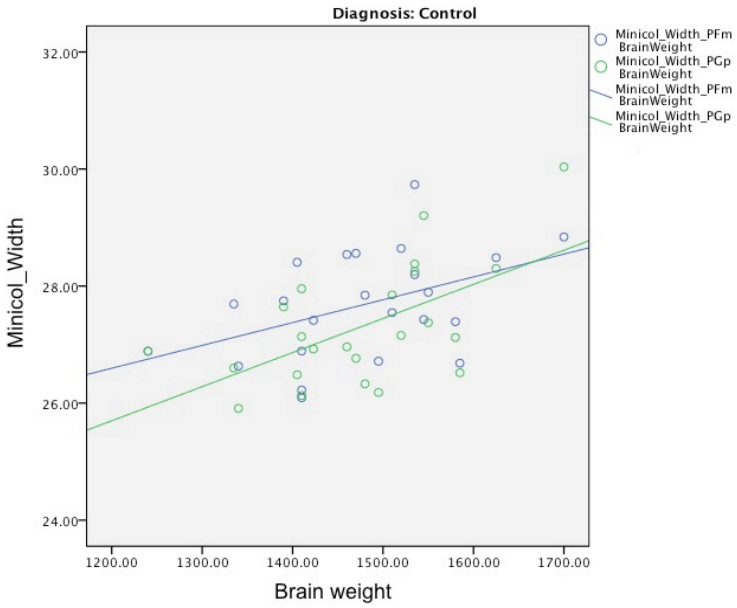
Post-hoc Analyses: Tissue Variables

An independent samples t-test was performed to look at the differences between the diagnoses on measures of age, brain weight, brain pH and PMI. A Levene's Test for Equality of Variances did not reach significance for any of these variables, indicating that equal variances were assumed between diagnoses. Brain pH was significantly different between diagnoses (t=2.385, df=46, p=0.021). Age in patients was significant on a Shapiro-Wilk test of normality and was analyzed using the Independent-Samples Mann Whitney U Test; this variable did not reach significance on a test of differences between diagnoses.

Correlations

We used the Pearson's two-tailed correlations test, as well as the nonparametric Spearman's correlation test, to further investigate the data. In controls, brain weight was positively correlated with minicolumn width in PFm (Pearson correlation=0.441, p=0.035, n=23) and PGp (Pearson correlation=0.575, p=0.003, n=24) (see Figure 8), as well as spacing in PGp (Pearson correlation=0.646, p=0.001, n=24). Spacing in PFm was negatively correlated with brain pH (Pearson correlation=-0.425, p=0.043, n=23); PMI and brain pH were positively correlated (Pearson correlation=0.472, p=0.020, n=24).

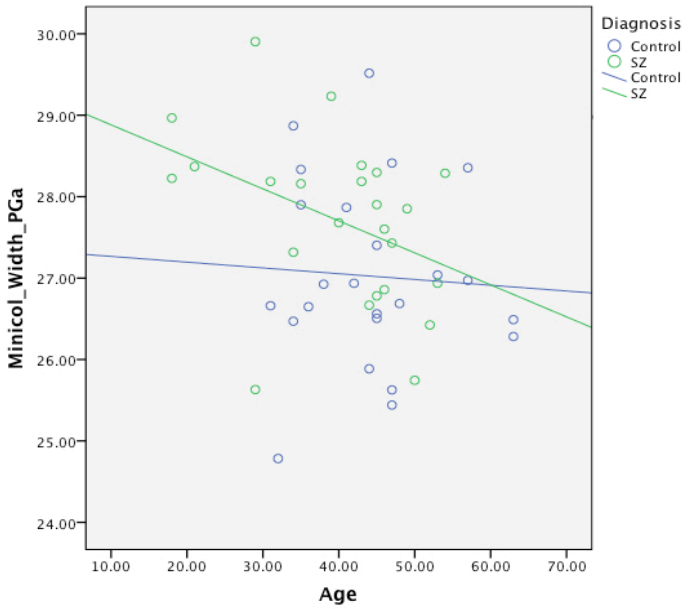
Figure 8. Brain weight and minicolumn width



The relationship of minicolumn width with changing brain weight in area PFm (blue) and area PGp (green) in controls.

In patients, age was negatively correlated with minicolumn width in PGa (Spearman's rho=-0.455, p=0.025, n=24) (see Figure 9). Age was also positively correlated with age of onset (Spearman's rho=0.417, p=0.043, n=24), duration of illness (Spearman's rho=0.771, p=0.000, n=24), and medication (Spearman's rho=0.590, p=0.002, n=24). Minicolumn width in PFm was positively correlated with brain pH (Pearson correlation=0.516, p=0.012, n=23). Brain pH and brain weight were also positively correlated (Pearson correlation=0.569, p=0.004, n=24).

Figure 9. Age and minicolumn width



The relationship of minicolumn width with changing age in area PGa; this relationship was significant in patients (at p<0.05).

Discussion

Differences between patients and controls

There was no overall difference in minicolumn width or spacing between controls and patients; however, post-hoc tests indicated that there was an increase in width in area PGa in patients compared to controls. There was also a difference in minicolumn width between PGa and PFm within controls, where PFm was wider; this is in contrast to the patient population, where there were no differences between the regions. These findings indicate that it is region PGa that has different microstructure between patients and controls, and that this difference may be due to a change in controls, and not a change in patients. This may indicate that patients exhibit a more homogenous brain structure in the IPL.

As minicolumns form during development[3], alterations in the minicolumnar structure would signal an alteration in neurodevelopment in schizophrenia. This is a

theory that has been suggested previously, and is supported by a wide variety of research[22-25]. Most commonly, altered neurodevelopment has been suggested to occur in the prefrontal cortex[26-30]. In the current study, the cytoarchitectonic parcellation suggested by Caspers et al[20] was used; this parcellation was developed through the use of an observer-independent scanning methodology to identify probabilistic maps, which were then normalized to the Montreal Neurological Institute (MNI) standard brain. This parcellation identified seven structurally different regions in the inferior parietal lobe; broadly speaking, differences between these regions lay at the level of the cytoarchitecture, and were described using width of the cortical layers, descriptions of pyramidal neuron size and shape, density of neurons, and to a lesser extent, anatomical landmarks[20]. This level of organization is differentiated very early in the lifespan[31]; the fact that the IPL may be more homogenous in patients, as is suggested by findings from the current study, may indicate that this process of differentiation is abnormal in schizophrenia.

In both controls and patients, spacing in area PFm was increased compared to PGa and PGp. This indicates that the space between minicolumns, which is mostly comprised of neuropil, including dendritic spines and synapses, changes in a similar way in both controls and patients. In controls, there was a difference in width between PFm and PGa; this is likely due to the increased spacing. However, in patients, there was no difference in width between the areas; therefore, the size of the minicolumn itself does not change, although the spacing between the cell-dense cores of the columns (included in the minicolumn width measurement) does change. If there is a change in the spacing component of the minicolumn but no change in the whole minicolumn, there must also be a change in the cell-dense component of the minicolumn in order to accommodate the change in spacing. This indicates that the minicolumn cell-dense core may be wider

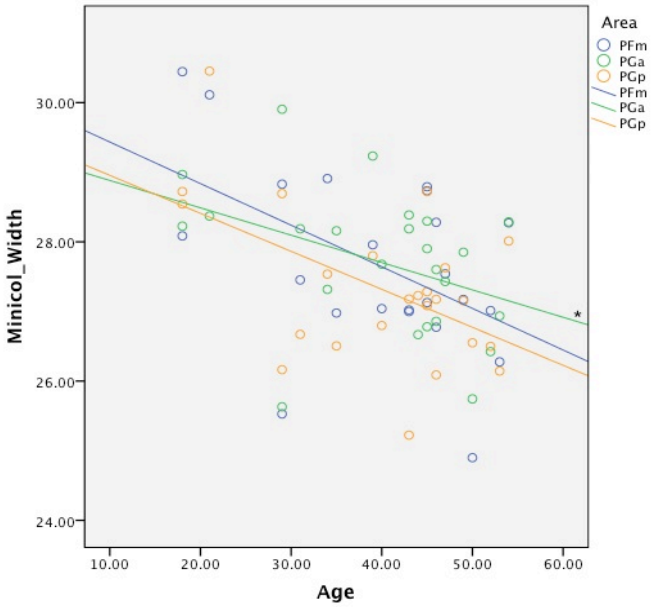
in PG, which is consistent with Casper et al's[20] description of the region, as well as by my own findings in the current study. The similar relationship in both diagnostic groups may indicate that changes in neuropil may not be a defining characteristic of schizophrenia; this has previously been suggested[32].

The finding of similar spacing in controls and patients firstly provides support for the structural differentiation of the IPL into these cytoarchitectonic categories. The difference in spacing between PFm and PG could be indicative of a functional significance; more spacing implies increased neuropil and connections[4], and therefore the decreased spacing in PG could indicate that this area contains less neuropil than PFm. The angular gyrus, which correlates to area PG, has been likened to a multisensory hub by a recent review[33], with functions ranging from social cognition and semantic processing to memory retrieval and attention shifts. The supramarginal gyrus, which area PFm is close to, has been suggested to be involved in memory retrieval facilitated by miming actions[34]. The fact that this spacing trend is seen in both patients and controls implies that this is an inherent feature of the IPL, and may not be related to the functional differences in schizophrenia.

As patients age, minicolumns become thinner in PGa; this relationship also approached significance in both PFm ($p=0.058$) and PGp ($p=0.076$) (see Figure 10). No association was found between minicolumn width and aging for controls. There was no correlation between spacing and age in either patients or controls. This supports previous findings that there is a decrease in gray matter volume with age in schizophrenia[35,36]. These are however in opposition to results previously found on minicolumn width in other areas: Di Rosa et al[21] found that as schizophrenia patients aged, minicolumn width increased in the fusiform cortex; Chance et al[1] found that in the

planum temporale, there were no changes in minicolumn width in patients at all. This was in contrast to controls, where there was a negative correlation between minicolumn spacing and age. These opposite results may be due to the different developmental trajectories of the parietal and temporal lobes: the parietal lobe, and particularly the IPL, may be one of the last brain areas to myelinate[8], and the cytoarchitecture may not mature until late childhood[37]. This is supported by a study of gray and white matter ratios in the parietal cortex, where the relationship of gray matter to white matter was variable until around age 23, at which point it became stable[38]. Studies of development have shown that gray matter in the IPL reaches full maturity around age 8.5 years, while some areas of the temporal cortex may mature earlier[39]. Since the planum temporale is located within the temporal cortex, this region likely matures at a different time than the inferior parietal lobe[39]. This temporal difference in maturation may indicate that there is a vulnerable period during development during which schizophrenia deviates from the norm; this has been suggested previously[40]. This has significant implications for changes in the inferior parietal lobe, as the IPL has many important roles in the development of concept of self, sensory integration, and executive functioning, functions that are notably altered in schizophrenia[8]. Given that the pattern of development is different[39], and that this study shows that the aging pattern in the IPL is different, as patients with schizophrenia age, they may become increasingly more impaired on these measures. Although some studies have shown that there is no deterioration in clinical presentation[41], others have shown that there is cognitive decline in schizophrenia[42].

Figure 10. Aging trajectories in patients



*The relationship of minicolumn width with age in PFm (blue), PGa (green), and PGp (orange); * denotes the region where this relationship was significant, although the trajectory is similar in all three areas.*

Altered aging trajectories have been shown in schizophrenia previously[43-45]; although there is little literature on trajectories in the microstructure, whole-brain analyses have shown that while controls show a curved decrease in gray matter volume over time, the trajectory in patients is linear[44]. Additionally, there have not been many studies of aging in parietal tissue in schizophrenia; however, the results found here, in the linear decrease of minicolumn width with age, seem to support the dichotomy in aging trajectories. Several authors have supported an alteration of the neurodevelopmental hypothesis to account for these findings, producing the progressive neurodevelopmental model. This theory posits that although there is an original developmental alteration that causes the initial brain changes that bring about schizophrenia, the brain later deviates even more from healthy subjects by following a different trajectory of aging[40].

As brain weight increases in controls, minicolumn width increases in areas PFm and PGp, as does spacing in area PGp; there are no significant changes in width or

spacing with brain weight in patients. The increase in width and spacing in controls may be due to an increase in neurons; since neuron density was not analyzed in the current study, it is unclear whether there was a change in neuron number. However, since the measure of minicolumn width is comprised of both a cell-dense core and a cell-sparse surrounding neuropil space, it is a logical hypothesis that this increase in minicolumn width with increasing brain weight is a function of increased cells and connections. Unpublished data on neuron density performed on this same sample by coworkers in area PFm indicates that there is no change in density in controls with increasing brain weight (East, unpublished data). This indicates that if there is an increase in neuron number, it is accompanied by a concomitant increase in brain volume with increasing brain weight in controls; however, this relationship is not seen in patients. This provides further support for the theory that there are alterations in the neuronal landscape in this region in schizophrenia.

Clinical variables

There were no significant relationships between minicolumn width or spacing with either age of onset or illness duration. However, age of onset is often highly stratified according to gender[46], and the current sample only included males. Although this does not preclude the existence of relationships between age of onset and brain changes, as has been suggested[47], this was not found in the male subjects in the current study. Since an earlier age of onset generally indicates a more severe course of illness[48], not finding any correlation with age of onset or illness duration and brain changes may indicate that this more severe course is not reflected in brain changes in the IPL in patients. Additionally, this lack of a relationship may indicate that the brain changes that

are seen in schizophrenia are inherently part of the disease, and do not depend on length of illness, or at what age the illness began.

Both age of onset and illness duration were however related to age; as age increases, the age at which the illness begins increases, and the duration of illness increases. This means that older patients tend to have both a later age of onset, as well as a longer duration of illness. Longer illness duration does not necessarily indicate more severe course of disease[5], although increased brain changes have been found with a longer duration of illness[15,49]. This may seem contradictory at first. However, research has shown that individuals who have an earlier age of onset tend to have a more severe disease, with more debilitating symptoms[50]; these individuals tend to have a worse prognosis[51]. Having a more severe course of illness would make it more difficult to have meaningful social interactions, hold down a job, or be self-sufficient; it has also been shown that those who have a worse prognosis, such as is indicated by an earlier age of onset, tend to have more severe cognitive symptoms[50]. There is a greater risk for suicide in this group[52]. Individuals who have a later age of onset, on the other tend, may have a better prognosis and less severe course[53]; such individuals may therefore tend to have longer lifespan, which would lead to a longer duration of illness.

As age increases, the amount of medication also increases. As medication in this case was indicated by lifetime fluphenazine equivalents, it makes sense that with increasing age, there is an increase in the amount of lifetime medication. This is especially true given that increasing age indicates longer illness duration in this study. Those who are diagnosed with schizophrenia are generally medicated directly after or during the first-episode[54], and this medication may, and often does, continue throughout the course of the disease[55]. It is unexpected that medication did not therefore correlate

with illness duration; however, given that this sample included several patients who experienced early age of onset, perhaps this influenced the results. There were 12 schizophrenia subjects who had an onset earlier than age 18, which is generally considered to be early-onset schizophrenia[51]. As earlier age of onset is associated with a more severe course of disease[53], and such people may not be as responsive to treatment[40]; these people may be exposed to higher levels of medication in an effort to increase treatment response[54]. These factors may have influenced how the amount of lifetime medication dosage correlated with illness duration. Also, given that this brain collection was collected between 1999-2005, and that the mean age at death of patients was 40 years of age, it can be inferred that many of these patients had onset around 1979-1985. The practice of prescribing medication has significantly changed since then: in 1998, the recommended dosage during an acute episode was 400-600 chlorpromazine equivalents, yet there was a substantial number of patients being prescribed several times this amount, especially of the "high-potency" antipsychotics[54]; in 2000, the recommended dosage for treatment of an acute episode was 300-600 chlorpromazine equivalents[56].

Studies looking at insight into the illness have mainly been interested in associating insight with symptoms and impairment[57]; however, measures of insight can also be used to examine the level of cognitive reserve, which is a quantitative measure of how well people respond to brain pathology[58]. An increase in insight may indicate that patients are more responsive to treatment[59], have a higher IQ[16], and exhibit better executive functioning[60]. These traits are also characteristic of those with improved cognitive reserve[61]. In the current sample, spacing in area PFm was greater in those that had poor to fair insight into their condition, compared to those who had good insight. As mentioned, increased spacing indicates increased neuropil; as there was no change in

minicolumn width, perhaps this also indicates that those who have increased neuropil have decreased width of cell-dense columns, indicating fewer neurons. As decreased minicolumn width has been correlated with healthy aging, and, more severely, with dementia[62], the increased spacing in poor insight might imply a failure of neuroplastic processes. Such a theory has been proposed previously[1], and might contribute to the decreased insight of such patients, as mediated by a reduction in cognitive reserve.

Comorbidity

Overall, substance use was increased in patients: compared to controls, almost four times the number of patients smoked at time of death, twice the number of patients had a history, past or present, of alcohol use, and three times the number of patients had a history, past or present, of drug use. In patients, those who had a history of alcohol use more often also had a history of drug use and smoked at the time of death than those who did not have a history of alcohol use.

It is known that those with schizophrenia often use substances such as tobacco, alcohol, and drugs in order to help ameliorate both symptoms and medication side effects[63]; this high comorbidity is pervasive in the population[64]. Those who show comorbid substance abuse exhibit poorer outcome and may have an earlier onset of symptoms[65]. This was shown in the current study, as age of onset was earlier in those who had a history of drug use; drug use, especially cannabis, has been linked to earlier onset[66]. As details on exactly what kind of drug was being used were not available in the current study, the relationship found here may suggest that cannabis was the drug of choice.

In the current study, spacing in PFm was greater in those patients who had a history of alcohol use. Animal models have shown that alcohol use can cause neuronal loss[67], which may support this finding: with an increase in spacing but no change in overall minicolumn width, the cell-dense core must be decreasing concomitantly with the increase in spacing, indicating loss of neuron number or size.

Brain weight was higher in patients who had a history of drug use, whereas in controls brain weight was lower with a history of drug use. This differing relationship was unexpected, but may indicate that drug use has a different effect in a schizophrenia brain than it does in a healthy brain, and may be related to the theory of altered neuroplasticity in schizophrenia[1]. Cannabis use in schizophrenia has been linked to a higher premorbid IQ[66]; higher IQ has been correlated with increased cerebral and gray matter volume in children[68], which may mediate a relationship between IQ and brain weight.

There was also decreased minicolumn width in area PFm with drug use in controls; this is likely a reflection of the relationship between brain weight and minicolumn width that was found in controls: with increased brain weight, there was increased minicolumn width, significantly so in areas PFm and PGp. The relationship found here with drug use reverses this trend: decreased brain weight and decreased minicolumn width were both found with increased drug use. Decreased minicolumn width has been posited to occur in brain shrinkage[69], and that is implied here by the concomitant decrease in brain weight; this has been supported by findings of decreased brain size with cannabis use in adolescents[70].

An important fact to take note of in this interpretation is that information was not available on the different types of drugs that were used; previous research has shown that various drugs have different effects[63] and so it is difficult to detangle which effects are due to a particular drug. However, given the relationship between drug use and age of onset in schizophrenia, it may be a reasonable hypothesis that cannabis was highly represented as the type of drug used. Additionally, this analysis was performed so that all alcohol or drug use, either past or present, use or abuse, was counted as a positive sign of use. This was done to facilitate the statistical analysis, as the groups were too small for comparison otherwise. However, this choice does lose information in the process; with a larger sample, this sort of temporal information on the history and quantity of drug use would be very helpful.

None of the controls that were included in this study died by suicide, whereas six of the schizophrenia patients did die by suicide. There were several differences in patients between those who did and those who did not die by suicide: minicolumn width in all three regions was increased in those who did suicide, and they were also of a younger age, had a shorter duration of illness, and had a lower amount of lifetime medication. It has been reported previously that the risk of suicide is higher in patients, particularly after onset of symptoms[71]. Patients who die by suicide tend to be younger and male[72], have a later onset[73], and a shorter duration of illness[74]. Noncompliance with medication is also associated with suicide[75]. These findings are supported in the current study. The increased minicolumn width associated with suicide here may be a factor of the younger age of these subjects, or their shorter duration of illness.

Tissue variables: Brain pH and PMI

Brain pH was the only tissue variable that was different between patients and controls; pH was significantly higher in controls, at a mean of 6.68, compared to patients, at a mean of 6.52. Differences between patients and controls could potentially be due to this tissue effect. Brain pH may be the variable that is of most significance in determining tissue quality[76]; however, in the current study where a more robust[1] Nissl stain was used, this likely would not have had a strong effect on the results found.

In controls, there was a positive correlation between brain pH and spacing in area PFm; pH was also positively correlated with post-mortem interval (PMI). Brain pH has previously been shown not to be associated with PMI[77]; therefore, the correlation found here may be coincidental. However, as molecular studies have indicated that less mRNA is available from more acidic brains[78], the correlation with brain pH and spacing here may indicate that less acidic control brains have more neuropil and are less subjected to the acidosis that occurs with age.

In patients, brain pH was positively correlated with brain weight and minicolumn width in area PFm. Brain pH is said to decrease with age[77]. Therefore, the effect of changing brain weight and column width with brain pH may in fact be an effect of age.

Conclusions

The tissue and data included in the current study allowed for a multifaceted investigation of cytoarchitecture in schizophrenia and healthy subjects, with numerous findings. Minicolumn width was significantly different in one subarea of the IPL in schizophrenia. Changes with age in the cytoarchitecture were also different in schizophrenia. These results support a theory of altered neuroplasticity in

schizophrenia, which contributes to different changes with age in the disease. These findings also have implications for proposed altered neurodevelopment in schizophrenia, and taken together provide support for a progressive neurodevelopmental model of schizophrenia. Additionally, these results provide evidence of different aging trajectories in the parietal and temporal cortices, as well as implications for brain weight and cytoarchitecture in healthy subjects. The contribution of clinical variables, such as age of onset, illness duration, medication and insight is complicated by relationships with age and possibly gender; however, the data collected here contributes to the growing body of knowledge on these topics. The information provided on comorbid conditions led to deeper understanding of the data, and the many ways that alterations in cytoarchitecture can affect, or be affected by, the environment. Lastly, as is necessary in any study involving postmortem tissue, an understanding of the effects of tissue processing is critical to the validity of findings.

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Appendix C

Chapter Three: Minicolumn width in the inferior parietal lobe

Table 1. Details of slides and measurements in area PFm

Subject	Number of slides	Measurements per slide	Number of measurements
P1	5	2	10
P2	5	4	20
P3	5	2 from one slide, 4 from other 4 slides	18
P4	4	2 from 2 slides, 3 from 2 slides	10
P6	5	2	10
P7	5	2	10
P8	5	2	10
P9	5	2	10
P10	5	2	10
P11	5	2	10
P12	5	2	10
P13	4	2 from 2 slides, 3 from 2 slides	10
P14	5	2	10
P15	5	2	10
P16	4	2 from 2 slides, 3 from 2 slides	10
P17	5	2	10
P18	5	2	10
P19	5	2	10
P20	5	2	10
P21	5	2 from 4 slides, 1 from 1 slide	9
P22	5	2	10
P23	5	2	10
P24	5	2	10
P25	5	2	10
P26	5	2	10
P27	4	2 from 2 slides, 3 from 2 slides	10
P28	5	2	10
P29	5	2	10
P30	5	2	10
P31	5	2	10
P32	5	2	10
P33	5	2	10
P34	5	2	10
P35	5	2	10
P36	5	4 from 4 slides, 6 from 1 slide	22
P37	5	2	10

P38	5	4 from 1 slide, 6 from 2 slides, 7 from 1 slide, 11 from 1 slide	34
P39	5	2	10
P40	5	2	10
P41	5	2	10
P42	5	2	10
P43	4	2 from 2 slides, 3 from 2 slides	10
P44	5	2	10
P45	5	2	10
P47	5	2	10
P48	5	2	10

Table 2. Details of slides and measurements in area PGa

Subject	Number of slides identified as PGa	Number of slides included in analysis	Measurements per slide	Number of measurements
P1	7	7	1 from 7 slides, 2 from 3 slides	10
P2	11	10	1	10
P3	10	10	1	10
P4	10	10	1	10
P5*	7	6	1 from 2 slides, 2 from 4 slides	10
P6	6	6	1 from 2 slides, 2 from 4 slides	10
P7*	7	6	1 from 2 slides, 2 from 4 slides	10
P8	6	6	1 from 2 slides, 2 from 4 slides	10
P9	8	8	1 from 6 slides, 2 from 2 slides	10
P10	7	7	1 from 4 slides, 2 from 3 slides	10
P11	11	10	1	10
P12	8	8	1 from 6 slides, 2 from 2 slides	10
P13	8	8	1 from 6 slides, 2 from 2 slides	10
P14	11	10	1	10
P15	8	8	1 from 6 slides, 2 from 2 slides	10
P16	7	7	1 from 4 slides, 2 from 3 slides	10
P17	7	7	1 from 4 slides, 2 from 3 slides	10
P18	8	8	1 from 6 slides, 2 from 2 slides	10
P19	9	9	1 from 8 slides, 2 from 1 slide	10
P20	9	9	1 from 8 slides, 2 from 1 slide	10
P21	4	4	2 from 2 slides, 3 from 2 slides	10
P22	11	10	1	10
P23	12	10	1	10
P24	9	9	1 from 8 slides, 2 from 1 slide	10
P25	3	3	3 from 2 slides, 4 from 1 slide	10
P26	5	5	2 from 5 slides	10
P27	18	10	1	10

P28	9	9	1 from 8 slides, 2 from 1 slide	10
P29	6	6	1 from 2 slides, 2 from 4 slides	10
P30	13	10	1	10
P31	8	8	1 from 6 slides, 2 from 2 slides	10
P32	9	9	1 from 8 slides, 2 from 1 slide	10
P33	8	8	1 from 6 slides, 2 from 2 slides	10
P34	5	5	2 from 5 slides	10
P35	11	10	1	10
P36	13	10	1	10
P37	5	5	2 from 5 slides	10
P38*	8	6	1 from 2 slides, 2 from 4 slides	10
P39	11	10	1	10
P40	6	6	1 from 2 slides, 2 from 4 slides	10
P41	11	10	1	10
P42	10	10	1	10
P43	7	7	1 from 4 slides, 2 from 3 slides	10
P44	5	5	2 from 5 slides	10
P45	5	5	2 from 5 slides	10
P46	5	5	2 from 5 slides	10
P47	7	7	1 from 4 slides, 2 from 3 slides	10
P48	5	5	2 from 5 slides	10

*For these subjects, one or more of the slides initially identified as PGa were not included in the subsequent analysis, due to artefacts (ie. folds in the tissue, staining residue, holes or tears) and/or highly curved cortex in the tissue sample.

Table 3. Details of slides and measurements in area PGp

Subject	Number of slides identified as PGp	Number of slides included in analysis	Measurements per slide	Number of measurements
P1	8	8	1 from 6 slides, 2 from 2 slides	10
P2	14	10	1	10
P3	18	10	1	10
P4	10	10	1	10
P5	11	10	1	10
P6	14	10	1	10
P7	19	10	1	10
P8*	10	9	1 from 8 slides, 2 from 1 slide	10
P9	10	10	1	10
P10	20	10	1	10
P11	12	10	1	10
P12	9	9	1 from 8 slides, 2 from 1 slide	10
P13	14	10	1	10
P14	13	10	1	10
P15	14	10	1	10
P16	7	7	1 from 4 slides, 2 from 3 slides	10
P17	9	9	1 from 8 slides, 2 from 1 slide	10
P18	15	10	1	10
P19	20	10	1	10
P20	13	10	1	10
P21	8	8	1 from 6 slides, 2 from 2 slides	10
P22	12	10	1	10
P23*	9	8	1 from 6 slides, 2 from 2 slides	10
P24	14	10	1	10
P25	5	5	2 from 5 slides	10
P26	10	10	1	10
P27	12	10	1	10
P28	11	10	1	10
P29	6	6	1 from 2 slides, 2 from 4 slides	10
P30	14	10	1	10
P31	9	9	1 from 8 slides, 2 from 1 slide	10
P32	15	10	1	10
P33	13	10	1	10
P34	10	10	1	10
P35	9	9	1 from 8 slides, 2 from 1 slide	10

P36	13	10	1	10
P37	22	10	1	10
P38	4	4	2 from 2 slides, 3 from 2 slides	10
P39	11	10	1	10
P40	9	9	1 from 8 slides, 2 from 1 slide	10
P41	5	5	2 from 5 slides	10
P42	1	1	10	10
P43	9	9	1 from 8 slides, 2 from 1 slide	10
P44	9	9	1 from 8 slides, 2 from 1 slide	10
P45	8	8	1 from 6 slides, 2 from 2 slides	10
P46	17	10	1	10
P47	10	10	1	10
P48	13	10	1	10

*For these subjects, one or more of the slides initially identified as PGp were not included in the subsequent analysis, due to artefacts (ie. folds in the tissue, staining residue, holes or tears) and/or highly curved cortex in the tissue sample.

Final Comments

These studies have shown that there is an enormity of research and interest in changes in schizophrenia; there are a plethora of theories to account for all of these. In particular, the results here show support for a progressive neurodevelopmental basis to schizophrenia. From the discussions here, there is a multitude of theories that have been suggested to account for the microanatomical basis of this. Instead of subscribing to one or two of these, it may be more accurate to assume that there are multiple antecedents to these brain changes. For example, disinhibition by altered inhibitory cortical circuits may act at the same time as a reduction in neuropil to account for the extensive deficits that are seen in schizophrenia. These theories may be altered by age of onset and length of illness; it was noticeable in the current results that there were significant findings related to these clinical variables. The effect of medication in particular must not be discounted. These variables may all act together to produce the progressive changes that are seen in schizophrenia, heralded by altered neuroplasticity. This study has also shown that we still have much to learn about schizophrenia; although we are adding to our knowledge every day, it is clear that there are wide-ranging factors at work. Elucidating those factors, as well as their interrelationships, will help to clarify the changes that are seen in schizophrenia. This is especially true as many of the findings in the literature are inconsistent. Learning more about the techniques and methodologies will help to lessen experimenter-bias and keep focus on the results.