BRAIN ABNORMALITIES IN SCHIZOPHRENIA:

EVIDENCE FROM NEUROPATHOLOGICALLY SENSITIVE MRI TECHNIQUES

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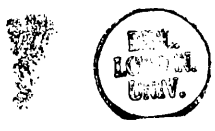

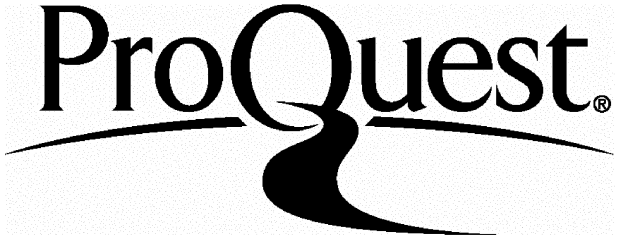

ABSTRACT

Post mortem and structural imaging studies in schizophrenia have reported macroscopic changes such as global and regional volume reductions but it has been more difficult to characterise the histopathological changes that underlie these abnormalities. The aim of this thesis was to further investigate white matter and cortical abnormalities in patients with schizophrenia using novel neuroimaging techniques, namely magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI) that are more sensitive to neuropathological changes in vivo than conventional MRI.

Studies of white matter abnormalities

Study 1

MTI was performed in 25 schizophrenic patients and 30 healthy controls to examine white matter using a region of interest (ROI) approach to measure magnetization transfer ratios (MTR). MTR was significantly reduced in bilateral temporal white matter but not in other regions of white matter in schizophrenic patients compared to controls. Clinical variables such as age, duration of symptoms, schizophrenic symptomatology and soft neurological signs did not predict the reduction in MTR. However, the pattern of correlations between the left and right frontal MTR values was different in the patients compared to controls suggesting that subtle abnormalities in interhemispheric connections may be present in schizophrenia. This study demonstrates that subtle white matter pathology restricted to the temporal lobes can be detected in schizophrenic patients using MTI and are most likely related to myelin and axonal abnormalities.

Study 2

DTI was used to investigate white matter abnormalities in 20 schizophrenic patients who were compared to 25 healthy controls. DTI changes were detected in the corpus callosum, specifically in the splenium but not the genu, suggesting that there may be a focal disruption of commisural connectivity in schizophrenia. In contrast, no DTI changes in other regions of white matter could be detected in a subgroup of these patients using a voxel-based analysis.

Study of cortical abnormalities

Study 3

Cortical abnormalities in the same group of patients and controls from Study 1 were examined using MTI. A voxel-based analysis revealed widespread MTR reductions in the cortex unrelated to volume reduction, particularly in the frontal and temporal regions, in the schizophrenic patients. The MTR reductions only extended into the white matter in the temporal lobes and not other regions. Reduced MTR in bilateral parieto-occipital cortex and the genu of the corpus callosum was associated with the severity of negative symptoms in the patients. These findings suggest that subtle neuropathological changes in the cortex can be detected in schizophrenia using MTI.

Together, the results from the three studies suggest that the detectable pathology in schizophrenia is predominantly cortical. The cortical changes are widespread but preferentially involve the fronto-temporal regions. It is likely that abnormal connectivity in schizophrenia is mainly related to cortical changes with little gross disruption of white matter tracts. These studies also illustrate the potential use of MTI and DTI to investigate the neuropathology of schizophrenia although methodological issues relating to their data acquisition and analysis should be carefully considered.

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THE EXTENT OF MY PERSONAL CONTRIBUTION

In accordance with the requirements of the University of London, the extent of my personal contribution to the work in this thesis is specified as follows:

During the period in which the work was carried out, I was employed as a full time researcher on a grant from the Wellcome Trust. The grant holder and my supervisor was Professor Maria Ron, Institute of Neurology. I was involved in the design of the studies in this thesis and collected all the data. I personally recruited and interviewed all the subjects. I also conducted all the psychiatric and neurological assessments for the studies. I performed the statistical analyses of the clinical and MRI data in all the studies. The GLMM analysis in Study 1 was done with the assistance of a statistician, Dr. Sarah Brocklehurst. The SPM analyses for Study 2 and 3 were done with guidance from Dr Mark Symms. The neuroanatomical protocol for the region of interest analysis on MRI was written with advice from Professor George duBoulay.

This thesis is entirely my original work and that no other person should be held accountable for its contents.

Jacqueline Foong

INTRODUCTION

Structural brain abnormalities have been extensively reported in schizophrenia but the neural systems involved have not been well characterised. Brain abnormalities detected on conventional magnetic resonance imaging (MRI) are gross by definition with loss of volume indicating a severe pathological process. More subtle abnormalities which may have functional significance cannot be detected by conventional MRI. This has led to the application of novel MRI techniques, such as magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI), which have the potential for providing more neuropathological information in vivo as they may be more sensitive to subtle or early neuropathological changes. These techniques have been mainly applied to neurological diseases and have only recently been considered as investigatory tools for research in psychiatric disease.

This thesis is divided into two main sections:

Section 1 contains a review of the literature.

Section 2 is comprised of three interlinked studies with the central purpose of further characterising the neuropathological abnormalities in vivo in patients with schizophrenia using novel MRI techniques. The first two studies investigated the white matter in patients with schizophrenia using MTI and DTI respectively. The third study examined cortical abnormalities in these patients using MTI.

CHAPTER 1

SCHIZOPHRENIA

Schizophrenia is a chronic and disabling disease of the brain which leads to severe psychiatric symptoms. Historically, the first description of the symptomatology was made by Kraepelin in 1896 who used the term 'dementia praecox' and included the clinical subtypes of catatonia, hebephrenia and paranoia. He considered 'dementia praecox' to be a degenerative disease of the brain progressing to a state of chronic deterioration which was distinguishable from manic depressive psychosis. Bleuler first introduced the term 'schizophrenia' in 1911 to mean the 'splitting' of psychic functions and defined the four crucial psychological mechanisms he considered to be involved, namely, ambivalence, autism, loosening of associations and flattening of affect.

A more extensive review of the prevalence, clinical features, aetiology or treatment of schizophrenia is outside the scope of this thesis and only a brief review focusing on the key points relevant to the studies reported in this thesis is presented here.

Prevalence

Schizophrenia is, in fact, a relatively common illness, with an estimated lifetime risk of about 0.5-1% (Torrey et al, 1987). Prevalence rates of schizophrenia range from 0.2-2% in different countries and these variations are likely to be due to the different diagnostic definitions of schizophrenia that have been used. The average incidence rate

is about 15-20 per 10,000 per year and appears to be generally similar throughout the world.

Course

The median age of onset is between 15 to 45 years, usually in the early 20s for males and late 20s for females. The frequency of schizophrenia in greater in males, being about twice as common as it is in females. In many patients, there is a prodromal phase prior to onset usually characterised by social withdrawal, loss of interest, decline in self care or unusual behaviour. Most studies in schizophrenia have suggested that the course is chronic although some patients experience relapses and remissions.

Clinical features

Schizophrenia is characterised by a constellation of distinctive symptoms that can relapse and remit and may occur against a background of chronic deterioration in psychosocial functioning in some patients. One of the first and most important phenomenological descriptions of schizophrenia was made by Schneider (1959) who described 'first rank' symptoms which he considered to be diagnostic of the illness in the absence of any obvious organic pathology. These symptoms consisted of delusional perception, thought alienation (insertion/withdrawal/broadcasting), thought echo, third person auditory hallucinations and motor/sensory passivity phenomena. However, it has been reported that almost 20% of chronic schizophrenic patients have never had these symptoms.

Schizophrenic symptoms were later further categorised with the distinction being made between positive and negative symptoms (Crow, 1980). Positive symptoms

reflect a distortion of normal functions whereas negative symptoms reflect a loss of normal functions. Positive symptoms occur during acute episodes of illness and include delusions, hallucinations, passivity phenomena (motor/sensory), thought disorder (loosening of associations), thought alienation and motor disturbance (stereotypies/mannerisms/catatonia). Negative symptoms are of a gradual onset and the commonly reported features are affective flattening (lack of emotional expression), alogia (poverty of speech), anhedonia, avolition, apathy and social withdrawal. Negative symptoms account for a significant degree of morbidity particularly affecting social and occupational functioning. However, it may sometimes be difficult to differentiate negative symptoms from the side effects of medications particularly the typical antipsychotics, environmental understimulation or depression.

Diagnostic classification systems in contemporary psychiatry have attempted to standardise the criteria for making a diagnosis of schizophrenia. This has implications for research as it is uncertain whether schizophrenia represents a single disorder of markedly variable expression or is part of a spectrum of clinically related disorders such as schizoaffective and schizotypal personality disorder. It is also uncertain whether schizophrenia can be clearly separated from other psychoses as there is increasing evidence that it shares genetic antecedents, clinical features and outcome with bipolar disorder. The Diagnostic and Statistical Manual of Mental Disorders (DSM) and International Classification of Diseases (ICD) have ensured a much narrower definition of the diagnosis than was previously used. The Fourth Edition of DSM (DSM IV) criteria for a diagnosis of schizophrenia includes positive and negative symptoms of which at least two have to be present for a significant part during a one month period and have persisted for at least six months. An additional

category in the diagnostic criteria in DSM IV relates to impaired social and occupational functioning following the onset of the illness.

Cognitive deficits

It is now well recognised that cognitive functioning in schizophrenia is impaired, particularly in the domains of attention, memory and executive functions based on cross sectional studies in chronic patients (McKenna et al, 1990; Crawford et al, 1993; Pantelis et al, 1997; Mellers et al, 2000). These findings raise the possibility that they may be related to the chronicity of the disease. However, other studies have been able to demonstrate a range of neuropsychological deficits on measures of attention, verbal memory, spatial memory and executive function in first episode patients (Saykin et al, 1994; Hoff et al, 1992; Hutton et al, 1998; Gur et al, 1998a). One longitudinal study comparing 20 first episode patients and 20 patients with chronic schizophrenia to a group of controls has found that neuropsychological performance worsened in some patients after two years which was related to progression in structural changes, particularly in the frontal lobes (Gur et al, 1998a).

Initial suggestions that Alzheimer's disease is commoner in schizophrenia than in the general population have been refuted by more recent studies (Arnold et al, 1998; Purohit et al, 1998). It is unclear why cognitive impairment in schizophrenia occurs but a number of possibilities have been raised. It has been postulated that cognitive impairment may be a manifestation of whatever substrate that underlies the disease, that the brain in schizophrenia may be more vulnerable to cognitive impairment in response to normal age-related neurodegeneration or that it may be a novel form of neurodegeneration (Harrison, 1999c).

Aetiology

The aetiology and pathogenesis of schizophrenia are still unclear. In the past, the psychological and social theories relating to psychodynamic, family or maternal dysfunction proposed to cause schizophrenia led to it being considered a 'functional' or non organic disorder. However, the response to antipsychotic medications and increasing evidence of brain abnormalities in schizophrenic patients have overshadowed these theories. Biological aetiological factors that have been implicated are genetic factors and brain abnormalities including structural, neuropathological and neurochemical changes.

Genetic factors

Family, twin and adoption studies have suggested that genetic factors affect the risk of developing schizophrenia. There are reports of an increased risk of schizophrenia in first degree relatives with family studies reporting a risk of 10% for first degree, 3-4% for second degree relatives and 40% for a child of parents who both suffer from schizophrenia. Twin studies have reported higher concordance rates for monozygotic twins than dizygotic twins (Shields and Gottesman, 1972). Earlier adoption studies (Heston, 1966) suggest that there is a higher incidence of schizophrenia in those born to schizophrenic mothers compared to controls. Further support comes from a study by Kety et al (1994) in adoptees with chronic schizophrenia which found the disorder exclusively in their biological relatives and not their adoptive relatives. The prevalence of schizophrenia was 10 times higher in the biological relatives of the schizophrenic adoptees than in the biological relatives of the control group.

The mode of transmission in schizophrenia is complex and has not yet been established. It is unlikely that a single dominant gene is involved since the concordance rate among monozygotic twins in not 100% and some people can carry the genotype for schizophrenia without developing the disease. It has been suggested that multiple genes of modest effect are involved and an environmental trigger may be necessary for clinical expression. Attempts to determine the mode of transmission from genetic linkage and association studies have produced conflicting results. Recent studies have provided support for a number of susceptibility loci that have been implicated in schizophrenia including regions on chromosomes 1q, 5q, 8p, 11q and 20q (Gurling et al, 2001). Further studies particularly in larger samples are needed to confirm these findings.

Brain abnormalities

The structural, neuropathological and neurochemical findings in schizophrenia are discussed in the next two chapters. Some of these findings provide strong evidence that neurodevelopmental factors play a significant role in the pathophysiology of schizophrenia.

CHAPTER 2

NEUROIMAGING FINDINGS IN SCHIZOPHRENIA

Schizophrenia has been considered a brain disease since the time of Krapelin (1896) although attempts to define or characterise the neuropathological abnormalities have proven to be elusive. Earlier post mortem studies were compounded by methodological problems which led to difficulties in replicating results. Research interest thus waned and it has only been the advent of neuroimaging techniques in the last 25 years, initially computerised tomography (CT) and subsequently magnetic resonance imaging (MRI), that has renewed interest in the search for abnormalities of brain structure and function in schizophrenia.

Methodological issues

It is important to consider that there may be difficulties in comparing the results from different studies using a particular neuroimaging technique such as MRI. Given that MRI scanning technology has changed over the past decade, the findings of more recent MRI studies may not be directly comparable to those of earlier ones. The differences in patient selection in the studies such as gender, parental socio-economic status, clinical subtypes, chronicity and medications across the studies may also make it difficult in interpreting the findings. Patients with schizophrenia are a heterogeneous population and as most neuroimaging studies in schizophrenia are cross sectional, it may not be possible to generalise the findings in one study to other schizophrenic populations. In addition, conventional MRI is sensitive in detecting gross pathological changes such as volume reductions or focal lesions but may not be able to detect more

subtle pathological changes. Many MRI studies have used a region of interest approach to study particular brain regions by manual delineation of their boundaries on the imaging data. More objective and accurate measurements have been possible recently with improved MRI acquisition and post processing techniques, such as volumetric analysis and the application of voxel-based morphometry.

The brain abnormalities in schizophrenia that have been reported from CT, MRI and magnetic resonance spectroscopy (MRS) studies are summarised below:

a. CT/MRI findings

Ventricles and brain volume

The early in vivo studies using invasive pneumoencephalography reported ventricular enlargement in patients with schizophrenia. This was later confirmed in the first computer assisted tomography (CT) study by Johnstone et al (1976) who compared 17 patients with chronic schizophrenia to normal controls. This led to renewed interest in the search of brain abnormalities in schizophrenia and the proliferation of CT and subsequent MRI studies. A review of CT findings in schizophrenia (Shelton and Weinberger, 1986) concluded that over 75% of studies reported enlarged lateral ventricles.

Recent meta-analyses (Lawrie and Abukmeil, 1998, Wright et al, 2000) of MRI studies have confirmed the presence of ventricular enlargement in schizophrenia. There has been a suggestion from some studies that ventricular enlargement is greater in male than female patients (Nopoulos et al, 1997). However, the majority of studies have a bias towards male subjects and a recent meta analysis did not find any significant

gender differences (Wright et al, 2000). The lateral ventricles in particular have been consistently reported to be enlarged in a recent review of MRI studies over the past twelve years (Shenton et al, 2001). The median increase in lateral ventricular size in both genders was reported to be 22% in another review of volumetric MRI studies (Lawrie and Abukmeil, 1998). MRI studies that have examined the subdivisions of the lateral ventricles suggest that the ventricular body has the greatest increases (Lawrie and Abukmeil, 1998; Wright et al, 2000). Small increases in ventricular volume in the temporal horns, particularly on the left side (Degreef et al, 1992; DeLisi et al, 1991; Shenton et al, 1992; Roy et al, 1998; Niemann et al, 2000) have also been reported. It is possible that this is related to a reduction in brain tissue around the temporal horn and further support comes from finding a correlation between reduced temporal grey matter on MRI and increased CSF volume of the left temporal horn (Suddath et al, 1989) in patients with schizophrenia. Small increases in ventricular volume in the frontal (DeLisi et al, 1991, Degreef et al, 1992) and occipital horns (Degreef et al, 1992; Vita et al, 1995) in schizophrenia have been less consistently reported.

Enlargements of the third and fourth ventricles in patients with schizophrenia have also been documented. In a recent review of MRI studies between 1988 and 2000, over two thirds examining the third ventricle reported positive findings of enlargement. In contrast, only one in five studies that examined the fourth ventricle reported enlargement (Shenton et al, 2001). It has been suggested that due to its proximity to the thalamus, the enlargement of the third ventricle may be related to reduced thalamic volumes which has been recently reported in patients with schizophrenia (Andreasen et al, 1994a).

A review of 50 MRI studies of whole brain volume found that only 22% (11 studies) reported significant differences between schizophrenic patients and controls (Shenton et al, 2001). The median reduction in whole brain volume across MRI studies has been reported to be about 3% (Lawrie and Abukmeil, 1998). There is also some suggestion that the reductions in brain volume may be more evident in a subgroup of patients with schizophrenia, namely those of childhood onset, which may be related to more severe genetic or environmental neurodevelopmental insults (Jacobsen et al, 1996). The wide individual variation in brain size in the general population, and confounding factors such as age, gender, ethnicity, social class and nutritional deficits need to be considered as they may contribute to the modest findings of changes in brain size in schizophrenia.

Neuroimaging studies in monozygotic twins discordant for schizophrenia have reported that the affected twin has larger ventricles (Suddath et al, 1990; Reveley et al, 1982). Family studies have also shown that schizophrenic patients have larger ventricles and smaller brains than their unaffected relatives (Weinberger et al, 1981; Sharma et al, 1998). However, relatives of schizophrenic patients have been found to have larger ventricles and smaller brains than controls who do not have a family history of schizophrenia (Lawrie et al, 1999; Sharma et al, 1998). These findings support the suggestion that there is a genetic vulnerability to schizophrenia. Ventricular enlargement has also been detected in first episode schizophrenic patients in a recent longitudinal study (Puri et al, 2001). The authors report progressive ventricular enlargement in 14 of the 24 patients after 8 months although there was also evidence of a reduction of ventricular size in the remaining patients which may have reflected improved nutrition or hydration following treatment.

Grey matter

The global reductions in cortical volume in schizophrenia have been reported by a number of studies and may account to a significant degree for the observed reduction in brain volume (Harvey et al, 1993; Zipursky et al, 1992; Lim et al, 1996a). A review of studies which controlled for intracranial volume and head size reported a reduction of the magnitude of 4% in global grey matter volume in schizophrenic patients (Hopkins and Lewis, 2000). Most studies have not found any gender differences in grey matter volumes in schizophrenia.

Neuroimaging studies in monozygotic twins discordant for schizophrenia have shown that the affected twin had reduced cortical volume by 3% (Noga et al, 1996) while there were no differences between the unaffected twin and normal controls. Other studies have found that schizophrenic patients and their schizotypal relatives have increased cortical sulcal volumes compared to healthy relatives and normal controls (Cannon et al, 1994; Honer et al, 1995).

Studies have reported that the greatest regional volume reductions in grey matter relative to global differences are particularly in the frontal (Sullivan et al, 1998; Schlaepter et al, 1994; Goldstein et al, 1999; Wright et al, 1999), temporal lobes (Pearlson et al, 1997; Lawrie and Abukmiel, 1998; Wright et al, 1999) and medial temporal structures such as the hippocampus (Suddath et al, 1990; Shenton et al, 1992) and amygdala (Breier et al, 1992; Marsh et al, 1994; Wright et al, 1999).

Other researchers have proposed that the structural abnormalities in schizophrenia can be anatomically characterised at a supra-regional level (Wright et al, 1999,

Sigmundsson et al, 2001) using 3D voxel-based analyses. In the study by Wright et al (1999), MRI images were segmented into grey matter and ventricular-CSF maps and multivariate statistics were applied to parcellated regions in grey matter. Significant reductions in grey matter within the fronto-temporal system in schizophrenic patients compared to controls were found supporting the suggestion that the pathology of schizophrenia involves disruption in distributed fronto-temporal neural connections. These findings have been confirmed in another study which reported fronto-temporal grey matter volume deficits, particularly on the left, in a group of schizophrenic patients with predominantly negative symptoms (Sigmundsson, 2001).

The regional volumetric abnormalities that have been reported in schizophrenia are briefly discussed below:

i. Temporal lobe

The majority of MRI studies examining the temporal lobes in schizophrenia, have reported reductions in the total volume of the temporal lobes (Shenton et al, 2001). These positive findings are more commonly reported in recent than earlier studies which may be attributed to improved methodology allowing more accurate volumetric measurements with the acquisition of thinner slices on MRI.

Specific regions in the temporal lobes have been examined in schizophrenia. In particular, the *medial temporal lobe structures* have been extensively studied. Reduction in the volumes of amygdala-hippocampal complex and parahippocampal gyrus have been reported in patients with chronic schizophrenia. Both bilateral (DeLisi et al, 1988; Marsh et al, 1994; Velakoukis et al, 1999) and left-lateralised volume

reductions of the amygdala-hippocampal complex, particularly in male patients (Barta et al, 1990 & 1997; Hirayasu et al, 1998; Shenton et al, 1992, Velakoulis et al, 1999; Woodruff et al, 1997b) have been documented. Volume reductions of the amygdala-hippocampal complex have also been found in first episode patients with schizophrenia (Lawrie et al, 1999; Velakoulis et al, 1999; Copolov et al, 2000). Interestingly, it has recently been demonstrated that bilateral reductions in amygdala-hippocampal volumes can also be detected in unaffected high risk subjects with first degree schizophrenic relatives (Seidman et al, 1999; Lawrie et al, 2001) suggesting that it may be a marker of genetic vulnerability to schizophrenia. However, others have questioned whether these abnormalities are specific to schizophrenia as they have also been reported in other psychiatric populations such as chronic or first episode patients with bipolar disorder (Altshuler et al, 1998; Hirayasu et al, 1998; Velakoulis et al, 1999).

Another region in the temporal lobe that has been studied in schizophrenia is the *superior temporal gyrus*. Grey matter volume reductions of the superior temporal gyrus in patients with chronic schizophrenia have been more consistently reported in studies that have selectively examined grey matter than in those which have evaluated grey and white matter volumes together (Shenton et al, 2001). Some studies have found that these abnormalities are predominantly lateralised to the left (Barta et al, 1990; Shenton et al, 1992). It has been demonstrated that volume reductions of the superior temporal gyrus and amygdala-hippocampal complex are highly correlated suggesting that these regions are functionally interrelated (Shenton et al, 1992). Grey matter volume reductions of the superior temporal gyrus have also been reported in first episode patients with schizophrenia (Hirayasu et al, 1998; Velakoulis et al, 1999)

but not in first episode patients with bipolar disorder (Hirayasu et al, 1998). Total and grey matter volume reduction of the right superior temporal gyrus have also been reported in adolescent onset schizophrenia which was positively correlated with the age of onset (Matsumoto et al, 2001). In addition, these abnormalities have been detected in unmedicated subjects diagnosed with schizotypal personality disorder (Dickey et al, 1999) suggesting that they may be specific to schizophrenia and its spectrum disorders. It is not known if the superior temporal gyral abnormalities are progressive or static. There is some preliminary evidence from a one year follow-up study of 11 neuroleptic naïve first episode patients that left superior temporal volume reductions may be reversible early in the illness (Keshavan et al, 1998a). Larger samples are needed to confirm these findings and may help to elucidate whether they are related to early treatment with neuroleptic medication.

The *planum temporale* has been investigated in schizophrenia as it is involved in language and speech processing. In normal healthy controls, there is a left greater than right asymmetry. In a review of seven MRI studies that have examined planum temporale volumes in patients with chronic schizophrenia, most reported a loss of left greater than right asymmetry (Shapleske et al, 1999). One study has also found these abnormalities in first episode patients with schizophrenia but not bipolar disorder (Hirayasu et al, 2000). It has been suggested that abnormalities of the planum temporale are related to heteromodal association cortex abnormalities including posterior superior temporal gyrus, dorsolateral prefrontal cortex and inferior parietal lobule that may be involved in schizophrenia (Pearlson et al, 1996).

ii. Frontal lobe

In a review of 50 MRI studies in schizophrenia, Shenton et al (2001) found that 60% reported positive findings. Most of these studies have examined the frontal lobe as one structure and reported a reduction in volume. It is known that the frontal lobe is highly differentiated with different subregions within the frontal lobe having separate functions. However, to date only a few studies have selectively examined subregions within the frontal lobe or differentiated between the grey and white matter. Some have reported significant volume reductions in prefrontal grey matter in schizophrenic patients compared to controls (Gur et al, 2000) but have not been supported by others (Baare et al, 1999). Other studies have found volume reductions of the middle frontal and orbitofrontal regions in patients with schizophrenia (Goldstein et al, 1999). Improved methodology in neuroimaging will allow better delineation and measurement of the subregions in the frontal lobes and help to clarify whether abnormalities of frontal lobe volumes are localised or generalised in schizophrenia.

iii. Parietal and occipital

Very few studies have examined the *parietal lobe* in schizophrenia although it is important in language processing (Mesulam 1990) which may be disrupted in these patients. It is highly lateralised with left greater than right asymmetry and the region that has been particularly associated with language processing is the inferior parietal lobule which is comprised of the supramarginal and angular gyri. In a recent review, nine out of 15 MRI studies examining the parietal lobe have reported total volume reductions (Shenton et al, 2001). Only a few studies have examined the subdivisions of then parietal lobe. Volume reductions in the supramarginal gyrus (Goldstein et al, 1999) or inferior parietal lobule (Schlaepter et al, 1994; Frederikse et al, 2000) have

been reported in conjunction with volume reductions in the prefrontal cortex. Recent studies have also reported a reversal of normal left greater than right asymmetry in the angular gyrus (Niznikiewicz et al, 2000) and inferior parietal lobule (Frederikse et al, 2000) specifically in male schizophrenic patients. These findings need to be confirmed in further studies of the parietal lobe, particularly examining the inferior parietal lobule, in patients with schizophrenia. A recent interesting MRI study in a group of patients with severe early onset schizophrenia who were studied over a 5 year period has reported that the earliest cortical deficits were detected in the parietal regions which later extended to the temporal and dorsolateral prefrontal regions (Thompson et al, 2001).

Only a few studies have examined the *occipital lobe* in schizophrenia but the findings are inconsistent (Shenton et al, 2001). Reports of volume reductions (Zipursky et al, 1992; Andreasen et al, 1994b) have not been confirmed by others (Schlaepter et al, 1994; Jernigan et al, 1991). There is little evidence to date to suggest that the occipital lobes are significantly involved in the neuropathology of schizophrenia.

White matter

Most volumetric studies have reported normal white matter volumes in contrast to widespread grey matter volume reductions (Harvey et al, 1993; Zipursky et al, 1992; Lim et al, 1996a). Some MRI studies have found total volume reductions in white matter (Cannon et al, 1998) whilst others have reported regional volume reductions, specifically in frontal white matter in patients with chronic (Buchanan et al, 1998) and early onset (Palliere-Martinot et al, 2001) schizophrenia. There is also some evidence that subtle abnormalities in white matter may be detected in schizophrenia despite

white matter volumes being preserved. Focal signal hyperintensities on MRI in white matter have been detected, particularly in the frontal lobes, in schizophrenic patients compared to controls, although the precise pathological significance of these abnormalities is uncertain (Persaud et al, 1997).

Particular attention has been given to specific white matter structures, namely the *corpus callosum*, in schizophrenia as it has been suggested that information processing deficits observed in patients with schizophrenia may be related to a disruption in interhemispheric connections largely mediated by the corpus callosum. Structural MRI abnormalities of the corpus callosum in schizophrenia have been documented including changes in length (Woodruff et al, 1993) and thickness (Nasrallah et al, 1986; Woodruff et al, 1993) although they have not been supported by others (Brown et al, 1986). Some investigators have reported gender differences with findings of reduced corpus callosum areas in female but not male patients with first episode schizophrenia (Hoff et al, 1994) though this has not been confirmed by others. A meta-analysis (Woodruff et al, 1995) has established that the majority of MRI studies have found a reduction in the area of the corpus callosum in schizophrenic patients compared to controls although little is known about the pathology or the functional significance of this structural abnormality.

Other brain structures

i. Thalamus

Reductions in thalamic volume in schizophrenic patients have been suggested by several MRI studies (Andreasen et al, 1994a; Buchsbaum et al. 1996; Staal et al, 1998) but have not been confirmed by others (Portas et al, 1998; Lawrie et al, 1999). This

may be due to methodological difficulties in the measurement of the thalamus on MRI in earlier studies as the grey-white matter boundaries are not clearly distinguishable. However, recent improved imaging techniques (Magnotta et al, 2000) may help in the evaluation of the thalamus in schizophrenia. A recent meta analysis of post mortem and MRI studies indicate a small but significant reduction in thalamic size in schizophrenic patients compared to controls even after adjustment for brain size (Konick et al, 2001). Additional support comes from PET studies which have shown reduced metabolism bilaterally in the mediodorsal nucleus of the thalamus in patients with schizophrenia that may indicate loss of functional input to the prefrontal cortex (Hazlett et al, 1999). Reductions in thalamic volume have also been reported in untreated patients with first episode schizophrenia (Gilbert et al, 2001; Gur et al, 1998b) and in unaffected high risk subjects (first and second degree relatives with schizophrenia) (Staal et al, 1998; Seidman et al, 1999; Lawrie et al, 2001). It has been proposed that the thalamus is critical in the neuropathology in schizophrenia as a disruption of the cortico-cerebellar-thalamic-cortical circuit leads to 'cognitive dysmetria' or a failure to coordinate the processing and retrieval of information which may underlie the cognitive impairment in these patients (Andreasen, 1996). One study however, has suggested that thalamic volume may be related to exposure to neuroleptic medication. In this study, thalamic volume was found to be reduced in neuroleptic naïve patients but not in previously treated patients and was positively correlated with the dose of neuroleptics (Gur et al, 1998b). Future studies using improved imaging techniques are therefore needed to further evaluate the thalamus in schizophrenia.

ii. Basal ganglia

The basal ganglia structures (caudate, putamen, globus palladus) have been of interest in schizophrenia because of the dopaminergic inputs into the striatum, response to neuroleptic medications and the importance of these structures in cognitive, sensory and motor processing. Conflicting findings of basal ganglia volumes on MRI have been reported in schizophrenia. Some studies have reported increased volumes of the caudate (Breier et al, 1992; Frazier et al, 1996), putamen (Shihabuddin et al, 1998; Jernigan et al, 1991; Elkashef et al, 1994) and globus pallidus (Elkashef et al, 1994; Frazier et al, 1996) whilst others have reported no differences (Corey-Bloom et al, 1995; Woods et al, 1996). Some investigators have found the increase in basal ganglia volumes to be related to illness duration and exposure to medication in their patients (DeLisi et al, 1991; Swayze et al, 1992). Studies in first episode patients with schizophrenia have reported increased caudate size at 1-2 years follow-up which was associated with earlier age of onset (Chakos et al, 1994) and dose of conventional neuroleptic medications (Chakos et al, 1994; Keshavan et al, 1994b). There is also some suggestion that patients on atypical neuroleptic medications, which have less affinity for D2 receptors than conventional neuroleptics, do not demonstrate an increase in the volume of basal ganglia structures (Chakos et al, 1995; Gur et al, 1998b). Interestingly, more recent studies in neuroleptic naïve patients with schizophrenia (Keshavan et al, 1998b; Shihabuddin et al, 1998; Corson et al, 1999) have reported reduced caudate volumes suggesting that basal ganglia abnormalities may be present prior to the use of medications although the specificity of these findings needs further investigation.

iii) Cerebellum

Very few studies have specifically examined the cerebellum in schizophrenia. Reduction in cerebellar volume has been reported by some investigators (Andreasen et al, 1994b, Katsetos et al, 1997) although others have failed to confirm this after controlling for height and total brain volume (Sharma et al, 1998). Cerebellar volume changes have not been found to be related to gender (Andreasen et al, 1994b; Nopoulos et al, 1997). It has been considered by some that schizophrenia may result from a disruption of the cortico-cerebellar-thalamic-cortical-circuit that is involved in motor and cognitive functioning (Andreasen et al, 1999) and therefore further studies are needed to evaluate the role of the cerebellum in schizophrenia.

b. MRS findings

Conventional MRI can delineate the volume in grey or white matter but is unable to describe the microscopic composition. White matter consists of axons surrounded by myelin sheaths which are comprised of myelin and glia. Grey matter consists of neuronal cell bodies, their synaptic, dendritic and axonal extensions, supported by a framework of glia. Magnetic resonance spectroscopy (MRS) is a MRI technique that is able to detect neurochemical changes in the brain. Phosphorus MRS allows the measurement of brain membrane phospholipids and high energy phosphate metabolism and has been applied in the study of schizophrenia. The first phosphorus MRS study in drug naïve first episode schizophrenic patients reported decreased phosphomonoesters and increased phosphodiesters in the frontal lobes consistent with decreased synthesis and increased breakdown of membrane phospholipids (Pettegrew et al, 1991). These findings have subsequently been confirmed by others (Stanley et al, 1995) suggesting that abnormal membrane phospholipid metabolism may occur in schizophrenia.

Other MRS studies in schizophrenia have applied proton MRS which detects hydrogen containing neurochemicals and is able to provide information about the neuronal and axonal integrity in vivo. The peak due to N-acetyl aspartate (NAA) is the largest signal in the proton spectrum, with creatine and choline also showing strong signals. Majority of proton MRS studies in schizophrenia have focused on NAA, an amino acid, which is considered to be a marker of neuronal or axonal integrity and is altered in disease states of neuronal damage such as acute and chronic multiple sclerosis (Miller et al, 1991), cerebral infarction (Bruhn et al, 1989; Fisher et al, 1992) and Alzheimer's disease (Kwo et al, 1994). Recent technical advances in MRS have also allowed the measurement of several other metabolites such as the neurotransmitters, glutamate and gamma-aminobutyric acid.

There are conflicting findings from proton MRS studies in schizophrenia. A number of studies have reported a reduction in NAA in the temporal lobes (Yurgelun-Todd et al, 1996a; Renshaw et al, 1995) and particularly the hippocampus (Maier et al, 1996; Bertolino et al, 1996; Lim et al, 1998) whilst others have reported negative findings (Buckley et al, 1994). The inconsistencies in MRS findings may be due to the different methodologies used with earlier studies measuring NAA/Creatine ratios and not absolute quantification of NAA. In addition, some studies were done in small samples and have not distinguished between grey or white matter in the volumes of interest examined.

An important study by Maier et al (1996) compared 25 schizophrenic patients to 32 age-matched controls and quantified concentrations of NAA, creatine and choline. The patients were found to have significant reductions in NAA, creatine and choline in the

left hippocampus. The NAA concentration was reduced by 22% in the patient group which lends support to histopathological reports of predominantly left sided hippocampal neuronal loss in schizophrenia (Falkai and Bogerts, 1986; Altshuler, 1987). It was also suggested that the reduction in creatine in these patients may be related to cell loss whilst the reduction in choline may reflect disruption of membrane or myelin components. Interestingly, significant reductions in hippocampal NAA have also been demonstrated in unaffected siblings of schizophrenic patients compared to controls (Callicott et al, 1998). These findings in conjunction with MRI findings of hippocampal abnormalities in first degree relatives of schizophrenic patients (Seidman et al, 1999; Lawrie et al, 2001) further supports the theory of a genetic susceptibility to schizophrenia

One MRS study (Lim et al, 1998) in 10 male chronic schizophrenics distinguished between grey and white matter in their assessment of NAA concentrations. They reported normal NAA concentrations in cortical grey matter in the presence of 18% volume reduction and in contrast, there was a 7% reduction in NAA in white matter without any volumetric change which may reflect a disruption of axonal connections. In addition, a study in first episode schizophrenic patients did not find any significant reductions of NAA in temporal grey matter (Bartha et al, 1999). Thus, the findings in these two studies suggest that NAA changes may be predominantly in white matter although this need to be confirmed in larger samples.

Reductions of NAA have also been reported in the frontal lobes, particularly in the dorsolateral prefrontal cortex, in patients with chronic schizophrenia (Bertolino et al, 1996). Neuroleptic naïve patients with schizophrenia have also been found to have

reduced NAA/Creatine ratios in the frontal and temporal lobes (Cecil et al, 1999). MRS has been used to investigate other brain regions in patients with schizophrenia, such as the thalamus in which reductions in NAA have also been reported (Omori et al, 2000).

Other MRS studies in schizophrenia have examined neurotransmitters in the brain such as glutamate. Glutamate, an amino acid, is a major excitatory neurotransmitter involved in synaptic transmission, plasticity and higher cognitive functions. It is involved in the connecting pathways of corticostriatal, thalamocortical and corticocortical association fibres. Some studies have reported abnormalities in glutamine, which is a precursor of glutamate, in patients with schizophrenia. Increased glutamine levels in the dorsolateral prefrontal cortex reflecting decreased glutamatergic activity have been reported in chronic schizophrenic patients (Stanley et al, 1996). Another small study compared 10 neuroleptic naïve patients with schizophrenia to controls and reported increased glutamine levels in the medial prefrontal region and anterior cingulate regions in the patients (Bartha et al, 1997). These findings are consistent with neuroimaging and post mortem findings of decreased glutamatergic activity in schizophrenia (Olney and Farber, 1995; Deakin et al, 1997) which is unlikely to be related to neuroleptic medications.

To date, the most consistent MRS findings in patients with chronic schizophrenia are reduced NAA in the hippocampus and to a lesser extent in the frontal and temporal cortex. These findings provide further indirect support for neuronal and axonal abnormalities in the temporal and frontal lobes in schizophrenia.

Clinical correlates of neuroimaging abnormalities

Attempts have been made to correlate brain abnormalities in schizophrenia with clinical variables. The main findings relevant to the studies in this thesis are as follows:

i. Soft neurological signs

Epidemiological studies have shown that subtle neurological signs such as dyspraxias, left handedness, poor coordination and social deficits present in childhood or infancy are associated with a diagnosis of schizophrenia in adult life (Jones et al, 1994) suggesting that abnormal brain development may be involved. The incidence of soft neurological signs (deficits in motor coordination, sensory integration or extrapyramidal) has been reported to be increased in patients with schizophrenia compared to healthy controls in cross sectional studies using standardised scales (Chen et al, 1995). There is also some suggestion that these signs may be progressive in schizophrenic patients (Chen et al, 2000b) or increased in non psychotic siblings compared to controls (Chen et al, 2000a) suggesting that they may be a marker of genetic vulnerability to schizophrenia and its progression. Soft neurological signs have been reported to correlate with prominent negative symptoms, poor psychosocial performance and cognitive impairment (Wong et al, 1997). The relationship between soft neurological signs and structural brain abnormalities has not been extensively investigated but it could be argued that structural abnormalities if present may be more severe in those with soft neurological signs. In general, studies have associated soft neurological signs with poor outcome and prognosis in schizophrenia.

ii. Schizophrenic symptoms

The earlier findings of ventricular enlargement being associated with negative symptoms (Crow, 1980) have not been confirmed in a more recent review of CT studies (Lewis, 1990). MRI studies attempting to relate negative symptoms to structural brain abnormalities have reported a range of findings. In some studies, the severity of negative symptoms in general has been reported to correlate with volumetric abnormalities in prefrontal grey (Baare et al, 1999; Gur et al, 2000) and white (Sanfilipo et al, 2000; Wible et al, 2001) matter, particularly in the orbital regions (Baare et al, 1999; Gur et al, 2000; Sanfilipo et al, 2000). One study found that specific negative symptoms, namely psychomotor poverty, correlated negatively with the volume of the left ventromedial prefrontal cortex (Chua et al, 1997). Further support comes from proton MRS findings that reduced NAA in the prefrontal cortex was correlated with the severity of negative symptoms in a group of chronic schizophrenic patients (Callicott et al, 2000). Other studies have also found negative symptoms to be correlated with left (Turetsky et al, 1995) or bilateral (Degreef et al, 1992) temporal volume changes and abnormalities of the corpus callosum (Gunther et al, 1991; Tibbo et al, 1998, Woodruff et al, 1997a).

It is important to consider that until recently, studies have tried to correlate numerical scores of clinical instruments measuring symptoms with global or regional volumes that require a priori assumption of a relationship between the two. It is only recently that voxel-based morphometry has allowed the exploration of these correlations without any a priori assumptions or the use of preselected regions of interest. Sigmundsson et al (2001) examined a group of schizophrenic patients with marked negative symptoms using voxel-based morphometry on MRI to determine whether

volumetric changes were related to clinical symptoms. They found that their patients had reduced grey matter volumes in the left superior temporal gyrus, left medial temporal lobe (including the hippocampus and parahippocampus), anterior cingulate and medial frontal gyri. White matter deficits were also present in left temporal regions and extended into the left frontal lobe. These findings suggest that patients with marked negative symptoms demonstrate left fronto-temporal deficits. Further evidence that negative symptoms may be related to underlying brain abnormalities has been provided by studies in neurochemical imaging which have found a negative correlation between striatal D2 receptor density and particular negative symptoms such as blunted affect, alogia (Martinot et al, 1994) or affective flattening and anhedonia (Knable et al, 1997). In addition, some functional imaging studies have demonstrated hypofrontality of regional cerebral blood flow in patients with negative symptoms (Liddle et al, 1992; Tamminga et al, 1992). Liddle et al (1992) found that psychomotor poverty correlated with reduced cerebral blood flow in the anterior cingulate and medial frontal lobe whilst Dolan et al (1995) reported abnormal modulation of anterior cingulate activation by apomorphine in patients with schizophrenia.

Severity of positive symptoms has been associated with volumes of mesiotemporal structures (Bogerts, 1993) and temporal horns (Degreef et al, 1992) although others have failed to find any correlation with overall temporal lobe volumes (Zipursky et al, 1994; Turetsky et al, 1995). Regional abnormalities of the temporal lobes such as superior temporal gyral volume reductions have been reported to be correlated with positive symptoms (Flaum et al, 1995) and with formal thought disorder (Shenton et al, 1992; Hollinger et al, 1999). Left superior temporal gyral deficits have also been found to be correlated with auditory hallucinations (Barta et al, 1990; Shenton et al,

1992; Marsh et al, 1997). Left planum temporale volume reduction has been found to be related to higher scores on the suspiciousness/persecution scale of the Positive and Negative Symptom Scale (PANSS) (Kwon et al, 1999) whilst planum temporale asymmetry has been reported to be associated with formal thought disorder (Petty et al, 1995; Rossi et al, 1994). One study has reported that increased grey matter volume in the basal ganglia was positively correlated with positive symptoms in a group of schizophrenic patients and suggests that this may reflect that patients with more severe positive symptoms are likely to receive greater doses of neuroleptic medication (Sigmundsson et al, 2001).

iii. Cognitive deficits

The pattern of cognitive deficits in schizophrenic patients is mainly characterised by deficits in memory and executive function thereby suggesting fronto-temporal localization in the brain. However, only a few studies have examined the correlation between structural brain abnormalities and neuropsychological deficits in schizophrenia. There are reports of reduced frontal and temporal lobe volumes being related to impaired neuropsychological performance (Andreasen et al, 1986; Gur et al, 1998a). More specifically, reduced volumes of the posterior superior temporal and parahippocampal gyri have been associated with poor performance on verbal memory, abstraction and categorization in patients with schizophrenia (Nestor et al, 1993). Volumetric changes in the prefrontal cortex may also be related to neuropsychological performance in schizophrenic patients as there are reports that reduced volumes correlate with poor performance on verbal recall, visual memory and semantic fluency (Baare et al, 1999) and greater volumes correlate with better performance on abstraction and attention measures (Gur et al, 2000).

Neuroimaging and neuropsychological abnormalities have been reported in first episode patients with schizophrenia which suggests that brain dysfunction may occur prior to clinical presentation (DeLisi et al, 1991; Hutton et al, 1998; Puri et al, 2001). Although some longitudinal studies suggest that these abnormalities are not progressive and do not correlate closely with any clinical features of the illness (DeLisi et al, 1991 & 1992), there is other evidence from a two year follow up study in patients with first episode schizophrenia that progression of structural changes may occur in some patients and is closely related to worsening of neuropsychological performance (Gur et al, 1998a).

CHAPTER 3

NEUROPATHOLOGICAL FINDINGS IN SCHIZOPHRENIA

Methodological issues

Neuropathological studies in the past encountered significant technical difficulties with tissue acquisition, inconsistent tissue handling and processing which may have led to tissue shrinkage. Another problem was that the case selection in earlier studies may have been unreliable as operationalised diagnostic criteria for schizophrenia was not widely used until the 1970s and control groups were inadequate. There is also a bias towards elderly subjects in these studies and therefore the effects of normal ageing on the brain cannot be ignored. In addition, the effect of medication may be a potential confounding factor in neuropathological studies.

Contemporary neuropathological studies have examined macroscopic and histopathological abnormalities in schizophrenia and are generally better designed and analysed. However, according to Harrison (1999c) in his review, some limitations persist, namely, small sample sizes, the failure to use stereological techniques, use of different histological techniques and the selection of different regions to be examined. These problems may make it difficult to compare findings across the different studies.

The main neuropathological findings relating to macroscopic, histopathological and neurochemical findings that have been reported in schizophrenia are summarised as follows:

Macroscopic findings

Ventricular enlargement and brain volume

Macroscopic abnormalities such as ventricular enlargement and loss of brain volume in schizophrenia have been demonstrated by post mortem studies. A number of studies have reported ventricular enlargement (Pakkenberg 1987, Bruton et al, 1990) and in one study, an increase of 33% in ventricular volume was detected in their group of patients (Pakkenberg et a, 1987). Some post mortem studies have found a selective increase in the volume of the lateral ventricles, particularly in the temporal horns of the lateral ventricles (Brown et al, 1986; Crow et al, 1989). This finding has been supported by MRI studies described in the previous chapter.

Significant global and regional brain volume reductions in schizophrenia have been reported by some post mortem studies (Pakkenberg 1987; Falkai et al 1988; Bogerts et al 1990). Pakkenberg et al (1987) reported a reduction of 8% in the overall volume of cerebral hemispheres in schizophrenic patients compared to controls.

Grey matter

The macroscopic findings of cortical volume reductions in schizophrenia have been reported by some post mortem studies (Pakkenberg 1987). Pakkenberg et al (1987) found volume reductions of 12% in the cerebral cortex and 6% in central grey matter but no loss in white matter volume in a group of patients with chronic schizophrenia. It has been suggested that grey matter volume reduction may be due to decreased cortical thickness (Brown et al, 1986; Pakkenberg et al, 1987; Selemon et al, 1995). The main post mortem findings of volumetric changes have been in the temporal and frontal lobes and are described as follows:

i. Temporal lobes

The macroscopic findings of the medial temporal lobe structures from post mortem studies are less consistently reported than MRI findings. This is most likely due to small sample sizes and differences in methodology for determining the anatomical boundaries and measurement techniques. Some studies have reported reductions in the amygdala-hippocampal volumes (Benes et al, 1991; Bogerts et al, 1985 & 1990; Falkai and Bogerts, 1986; Jeste and Lohr, 1989) which correspond to the loss in volume in the temporal horns of the lateral ventricles. Other post mortem studies have suggested that the abnormalities in medial temporal lobe structures in schizophrenia are lateralised to the left (Crow et al, 1989). Planum temporale size and asymmetry have also been investigated in post mortem studies and one study found a 20% volume reduction of the left planum temporale in schizophrenic patients compared to age-matched controls (Falkai et al, 1995).

ii. Frontal lobes

There are few post mortem studies that have found macroscopic abnormalities in the frontal grey matter. Small but not statistical significant reductions in cortical thickness (8%) of the prefrontal lobes have been reported (Selemon et al, 1998). More studies have examined the histopathological abnormalities in the frontal lobes which are described later.

White matter

Few studies have examined the white matter in schizophrenia and most post mortem studies have not found any alterations in white matter volumes. However, there has been particular interest in the corpus callosum in schizophrenia. The first post mortem

quantitative assessment of the corpus callosum by Rosenthal and Bigelow (1972) reported increased thickness of the corpus callosum in patients with schizophrenia compared to controls although this was not confirmed in subsequent studies (Brown et al, 1986). Histopathological changes in the corpus callosum have been reported and are discussed later.

Other brain structures

i) Thalamus

The post mortem macroscopic findings of thalamic abnormalities in schizophrenia have been conflicting. Some studies have reported reduced thalamic volumes (Pakkenberg, 1990 & 1992) but this has not been confirmed by others. There are also histopathological studies have examined specific regions of the thalamus which will be described later.

ii) Basal ganglia

Post mortem findings in the basal ganglia structures in schizophrenia have been inconsistent. Earlier studies did not find any abnormalities (Bogerts et al, 1985) but later studies have reported bilateral volume increases in striatum and globus pallidus (Heckers et al, 1991). This is supported by the findings from MRI studies discussed in the previous chapter.

Histopathological findings

It has been more difficult to characterise the histopathological changes that underlie the reported macroscopic abnormalities in schizophrenia. *Neuronal abnormalities* have been reported (Falkai et al, 1988; Jonsson et al, 1997; Benes et al, 1991 & 1998;

Akbarian et al, 1993a; Zaidel et al, 1997; Selemon et al, 1995 & 1998; Pakkenberg et al, 1993) although the findings have been inconsistent. Some have reported an increase in neuronal density in prefrontal cortex (Selemon et al, 1995 & 1998) and temporal lobes (Pakkenberg et al, 1993) including the hippocampus (Zaidel et al, 1997) whilst others have reported no change or reduced density in these regions (Jonsson et al, 1997; Benes et al, 1998; Akbarian et al, 1993b). Likewise, reports of a reduction in hippocampal and temporal neuronal cell number and size in schizophrenic patients (Benes et al, 1998, Zaidel et al, 1997) have not been confirmed by others (Pakkenberg et al, 1993). Neuronal abnormalities have also been detected in other brain regions such as the thalamus. In particular, a reduction in neuronal number of the thalamic dorsomedial nuclei which project to prefrontal cortex (Pakkenberg et al, 1990; Popken et al, 2000; Young et al, 2000) has been reported. Further studies are required to replicate these findings and to examine the different thalamic nuclei in schizophrenia.

Recent evidence suggests that neuronal abnormalities in schizophrenia may be subtle and localised to specific cortical layers. A reduction in interstitial neurons in superficial compartments of dorsolateral prefrontal cortex (Akbarian et al, 1996a) and reduction in neuronal size restricted to layer IIIc in the prefrontal cortex (Rajkowska et al 1998) have been reported.

Although the histopathological findings in schizophrenia have been inconsistent, a consensus is beginning to emerge from studies using stereological techniques. Thus, contrary to earlier reports of reduced neuronal density in the cortex (Benes et al, 1991; Falkai et al, 1988; Akbarian et al, 1993b), more recent studies using stereological techniques (Pakkenberg et al, 1993; Selemon et al, 1998) have found an increased in

neuronal density in the presence of cortical volume reduction. These findings have given rise to the suggestion that abnormalities in the neuropil, which is mainly comprised of neuronal processes such as dendrites and synapses, may be more significant than neuronal loss in schizophrenia and at least partly account for the cortical changes (Selemon et al, 1998; Glantz and Lewis, 2000). The reduced neuropil hypothesis suggests that the reduction in neuropil may be a key feature of cortical pathology in schizophrenia and may affect interneuronal connectivity (Selemon and Goldman-Rakic, 1999).

There is increasing evidence of *dendritic abnormalities* which may contribute to the disruption in neural circuitry proposed in schizophrenia. These abnormalities have been detected with Golgi staining, such as a reduction in spine density in layer III pyramidal cells in prefrontal cortex (Glantz and Lewis, 1997) and subicular apical dendrites of the hippocampus (Rosoklija et al, 2000). Other studies using indirect methods by measuring immunoreactivity have reported either a reduction (Arnold et al, 1991) or increase (Cotter et al, 2000) in microtubule-associated protein (MAP2), a dendritic marker, in the hippocampus suggesting that dendritic arborization may be altered in schizophrenia. Some studies have reported a reduction in synaptophysin, a synaptic protein marker, in the hippocampus (Harrison and Eastwood, 1998; Young et al, 1998) and dorsal lateral prefrontal cortex (Glantz and Lewis, 1997). Others have reported an increase in presynaptic growth-associated phosphoprotein (GAP-43) in frontal and occipital cortices, suggesting a disruption in synaptic organization (Perrone-Bizzozero et al, 1996).

Dendritic and synaptic changes in other brain regions have also been reported in schizophrenia. Alterations in the size (Uranova et al, 1996) and proportion of synapses (Kung et al, 1998) have been found in the caudate nucleus in schizophrenic patients though it is uncertain whether this is medication related. Synaptic degeneration in the thalamus has also been reported in other studies (Blennow et al, 1996).

Histopathological changes in *white matter* in schizophrenia have not been examined extensively. Earlier post mortem studies reported normal axonal density of white matter structures such as the corpus callosum (Nasrallah et al, 1983; Casanova et al, 1989). However, a more recent post mortem study has detected a reduction in number and density of axons in most areas of the corpus callosum in female schizophrenic patients (Highley et al, 1999). Abnormalities in prefrontal white matter such as a selective maldistribution of interstitial neurons have also been reported in schizophrenia (Akbarian et al, 1996a).

Neurochemical abnormalities

Changes in dopamine, serotonin, Gamma-aminobutyric acid (GABA) and glutamate have been implicated in schizophrenia and are briefly summarised here. The initial 'dopamine hypothesis' attributed schizophrenia to an excess of dopaminergic activity and was supported by the finding that positive symptoms respond to antipsychotic medications which block dopamine receptors. In addition, amphetamines can cause a paranoid psychosis similar to schizophrenia by increasing presynaptic dopamine release and inhibiting uptake. Earlier studies reported an increase in D2 receptors, particularly in the prefrontal regions suggesting that the prefrontal dopamine projections or their connections may account for mesocortical underactivity and

mesolimbic overactivity (Weinberger, 1987). However, the cloning of D1-D5 receptor genes and availability of ligands for PET and SPET studies have led to more recent PET studies which have not detected increased D2 receptor densities in unmedicated schizophrenic patients (Nordstrom et al, 1995). Recent PET and SPET studies have detected presynaptic dopaminergic abnormalities suggesting an excess of striatal dopamine in schizophrenia (Laruelle et al, 1996; Breier et al, 1997). Others have found that decreased dopaminergic function is associated with negative symptoms (Martinot et al, 1994; Knable et al, 1997).

Dopamine is no longer considered to be the only neurotransmitter involved in schizophrenia and other neurotransmitter systems have been implicated. Serotonin or 5-hydroxytryptamine (5-HT) is thought to be involved in schizophrenia because 5-HT agonist drugs such as LSD can cause psychotic symptoms. In addition, the atypical antipsychotics are potent 5-HT receptor antagonists. Changes in 5-HT receptor density such as a reduction in 5-HT2A receptors but an increase in 5-HT1A receptors in the prefrontal and temporal cortex have been reported (Harrison, 1997). These changes have been reported in post mortem unmedicated patients but have not been detected in vivo in young unmedicated patients using PET (Soares et al, 1999). This may suggest that these changes may be progressive during the course of the illness.

More recently, alterations in GABA and glutamate have also been reported in patients with schizophrenia. GABA is the principal inhibitory neurotransmitter in the brain and GABAmimetic agents can also cause psychotic symptoms. Prefrontal cortical reductions in inhibitory GABAergic interneurons (Beasley and Reynolds, 1997), cellular expression of the mRNA for the GABA transporter (Volk et al, 2000) and

glutamic acid decarboxylase (synthetic enzyme for GABA) (Ohnuma et al, 1999) have been reported. In contrast, others have reported increased cellular expression of the mRNA for GABA A receptors (Ohnuma et al, 1999) and increased GABA A receptor density in the cingulate cortex and hippocampal formation in post mortem tissue from schizophrenic patients (Benes et al, 1992 & 1996). These findings support the theory of a GABAergic deficit in schizophrenia.

There are fewer studies of glutamate systems in schizophrenia but there is some evidence that they may be involved. Glutamate is the principal excitatory neurotransmitter of the cortex and is contained in pyramidal cells. Apart from acting as a neurotransmitter, it is critical for neuronal migration and plasticity. There are 4 classes of glutamate receptors, namely alpha-amino-3 hydroxy-5-methy-4 lisoxazole propionic acid (AMPA), N-methyl-D-aspartate (NMDA) and kainate in postsynaptic sites and metabotropic (mGluRs) in presynaptic sites. The glutamatergic systems are complex and are thought to be involved in learning, memory and other cognitive functions. Phencyclidine, a non competitive antagonist of the NMDA (N-methyl D aspartate) type of post synaptic glutamate receptor, and other NMDA antagonists such as ketamine, are known to cause or exacerbate psychotic symptoms in normal subjects (Krystal et al, 1994) and schizophrenic patients (Lathi et al, 1995). Most findings of glutamate receptor abnormalities come from post mortem studies which have shown decreased binding to post synaptic kainate receptors in the limbic cortex, particularly the hippocampus (Kerwin et al, 1988) and increased binding to AMPA and NMDA receptors in the prefrontal cortex (Akbarian et al, 1996b) but it is not known whether such changes are present in vivo. Others have also reported reduction in the presynaptic receptor binding (Deakin 1989; Simpson et al, 1992) in prefrontal cortex.

There is also some indirect evidence of glutamatergic changes in schizophrenia from recent MRS studies (discussed in Chapter 2). It has been proposed that the abnormalities in glutamatergic transmission may underlie the cognitive deficits in schizophrenia (Keshavan, 1999).

A theory currently favoured is that it is not only the dopamine system but its complex interactions with other neurotransmitters, such as 5-HT, glutamate or GABA at the level of synapses or brain regions that are involved in schizophrenia. Despite the various neurochemical abnormalities observed in schizophrenia, their mechanisms in the pathogenesis of the illness have not been established and further studies are required to determine whether the neurochemical changes reflect histopathological abnormalities in schizophrenia.

Neuropathological effects of neuroleptic medications

It is known that neuroleptic medications affect neurotransmitters in the brain and can therefore confound neurochemical studies in schizophrenia. However, it may also cause structural changes in the brain which should be considered when interpreting structural imaging and neuropathological studies in schizophrenia. There are difficulties in studying the neuropathological effects of antipsychotic medications given that most patients have been prescribed neuroleptic medication at some time during their illness. Hence, this has been studied indirectly in several ways; comparing patients who have been drug free for a significant period though this assumes that medication effects are reversible, correlating with dose of life time medication assuming that there is a linear relationship, or comparing schjizophrenic patients with psychotic non-schizophrenic subjects such as patients with dementia or bipolar

disorder (Harrison, 1999b). In a review by Harrison (1999b), direct neuropathological investigations performed in animal studies (rats) have mainly used haloperidol and demonstrated neuronal and synaptic changes. Specifically, the studies reviewed have reported decreased neuronal packing density and possible increase in neuronal size in the striatum with sparing of the substantia nigra and cortical neurons. There are also reports of altered synaptic distribution in the striatum with increased proportion of asymmetrical and axodendritic synapses, which are mainly glutamatergic and excitatory, at the expense of asymmetrical and axospinous ones suggesting that antipsychotics produce an increase in inhibitory synapses. Thus, evidence to date would suggest that antipsychotics induce a subtle synaptic reorganization in the striatum and deep lamina of the frontal cortex. These findings have been reported with conventional antipsychotics but it remains to be determined whether atypical antipsychotics have similar effects.

There are very few studies in humans but an early study comparing chronic medicated patients with schizophrenia with a series of unmedicated ones suggested that histopathological changes, such as increased in neuronal size, were confined to the caudate nucleus and not detected in other regions (Jellinger et al, 1977). These changes are comparable to the findings in rats. Further support comes from some MRI studies that have reported striatal enlargement, particularly in the caudate, in schizophrenic patients on conventional antipsychotic medications but not in patients on atypical neuroleptic medications (discussed in Chapter 2).

There is little evidence from neuropathological studies to date to suggest that antipsychotics affect the hippocampus or cortical neurons. Furthermore, it does not

appear to promote the neuropathological changes of Alzheimer's disease (Harrison, 1999b). However, an interesting observation from some recent longitudinal studies in first episode schizophrenic patients is that in some patients, structural abnormalities on MRI such as lateral ventricular enlargement (Puri et al, 2001) and superior temporal gyral volume (Keshavan et al, 1998a) may be reversible early in the illness. These changes suggest that they may be related to early treatment with antipsychotic medication and further studies are needed to confirm this. The effects of other psychiatric medications which may be used in schizophrenic patients also need to be considered. This is in light of recent evidence from animal studies that long term administration of antidepressant medications and mood stabilisers such as lithium and valproate may promote neurogenesis in the hippocampus (Chen et al, 2000; Malberg et al, 2000) or have a neuroprotective effect by elevating cytoprotective protein levels (Manji et al, 2000).

Changes in cerebral asymmetry

Some investigators have suggested that the neuropathological abnormalities in schizophrenia are commoner in the left hemisphere (Brown et al, 1986; Crow et al, 1989). A review of neuropathological studies to date by Harrison (1999c) confirms that left sided pathology appears to be more frequently reported. It has been hypothesised that this can be explained by either genetic factors responsible for both the asymmetry and schizophrenia or that this is the result of abnormal brain development and lateralization in utero. However, others have not found any differences in laterality with respect to regional brain or ventricular volumes on MRI (Wright et al, 2000). Further studies are needed to clarify the cause and implications of cerebral asymmetry in schizophrenia.

CHAPTER 4

NEURODEVELOPMENTAL AND ABNORMAL CONNECTIVITY MODELS IN SCHIZOPHRENIA

Neurodevelopmental model

The defining lesions of schizophrenia have not been identified and many of the abnormalities described are unconfirmed or controversial. While some of the neuropathological findings are more suggestive of aberrant development than others, none can be attributed with certainty to development as opposed to postmaturational injury. Furthermore, it has been difficult to identify residual signs of abnormal development at post mortem (Arnold, 1999). However, there is some evidence from both clinical and neuropathological studies that neurodevelopmental factors play a significant role in the pathophysiology in schizophrenia. The neurodevelopmental model proposes that schizophrenia arises from abnormal brain development. However, it remains a challenge to researchers as to why the onset of the illness is typically in early adulthood. Two possible mechanisms have been suggested to explain the delayed onset of the clinical illness (Roberts et al, 1997). Firstly, it is possible that normal developmental events in brain maturation can moderate the behavioural expression of congenital neuropathology that affects specific neural circuits. Secondly, the delayed onset may be explained as resulting from an additional pathological process that occurs around the time of the onset of the clinical illness which is superimposed on the maldevelopment of the brain in utero.

The main clinical and neuropathological findings supporting the neurodevelopmental model in schizophrenia come from studies described below:

i. First episode studies

There is evidence from first episode studies in schizophrenia that structural brain abnormalities such as ventricular volume and decreased cortical volume (Bilder 1994; Nopoulos et al, 1995; Lim et al, 1996b; Zipursky et al, 1998) can be detected and do not appear to be correlated with the duration of illness. Furthermore, early neuropsychological deficits (Hutton et al, 1998; Gur et al, 1998a) in attention, memory and executive function detected in first episode patients with schizophrenia lend support to the neurodevelopmental model. Structural brain abnormalities, specifically reduction in amygdala-hippocampal and thalamic volumes, have also been detected in high risk unaffected individuals (Seidman et al, 1999; Lawrie et al, 2001; Staal et al, 1998) suggesting that at least in some patients, there may be a genetic vulnerability that may interact with neurodevelopmental factors in the development of schizophrenia.

ii. Longitudinal studies

Longitudinal studies are essential to determine whether structural brain abnormalities in schizophrenia are progressive during the course of the illness. The longitudinal data to date have been contradictory with some reporting that the pathological changes are not progressive (Vita et al, 1997) whilst others have found evidence of progression in at least in a subgroup of patients (DeLisi et al, 1997; Davis et al, 1998; Gur et al, 1998a).

iii. Neuropathological studies

The main neuropathological findings supporting the neurodevelopmental model in schizophrenia are reports of cytoarchitectural abnormalities. There are reports of ill defined lamination in the entorhinal cortex and disruption in the clustering of neurones in layer II and III with apparent heterotopic displacement of layer II-type stellate neurons deep in layer III (Jakob et al, 1986; Arnold et al, 1991; Arnold et al, 1997) suggesting an arrest of neuronal migration. Another possibility is that abnormalities in the neuropil disrupt the cortical cytoarchitecture (Selemon et al 1998; Glantz and Lewis, 2000). Others have found a reduction in neural cell adhesion molecule (NCAM) in the dentate gyrus and hippocampal region which affects migration during CNS development and also in synaptic rearrangement in maturity (Barbeau et al, 1995). The decreased expression on microtubule-associated protein 2 (MAP2) a developmentally expressed dendritic marker, in the hippocampus in schizophrenic patients (Arnold et al, 1991) also suggests that abnormal dendritic arborization may result from aberrant neurodevelopment. In addition, reports of abnormal white matter composition in frontal and temporal cortices and the selective maldistribution of interstitial neurons in prefrontal white matter which are remnants of the cortical subplate in schizophrenic patients (Akbarian et al, 1993a, 1993b & 1996a) have been interpreted as being consistent with faulty migration of subplate neurons which may lead to a disruption in neural circuitry.

iv. Absence of neurodegeneration

The natural history of schizophrenia is variable and it has been observed that some patients have a deteriorating course leading to dementia which suggests a progressive pathological process i.e. neurodegeneration. Cross sectional studies of chronically

hospitalised patients have demonstrated that the majority had severe cognitive decline (Davidson et al, 1995; Arnold et al, 1995; Harvey PD, 1999) that may resemble the cognitive deficits seen in Alzheimer's disease (Davidson et al, 1996). However, the biological substrates of these results have not been fully elucidated and the findings from structural imaging and neuropathological studies have been conflicting. The initial neuropathological findings of gliosis in schizophrenia (Stevens, 1982) have not been confirmed in subsequent studies (Roberts et al, 1987). It has generally been reported that the degenerative and atrophic processes such as neurofibrillary tangles, senile plaques, Lewy bodies, glial fibrillary acidic protein (GFAP) astrocytes have not been detected in the hippocampus, cortex or subcortical structures such as the thalamus or caudate using immunohistochemical techniques in neuropathological studies of elderly schizophrenic patients (Arnold et al, 1998 & 2000). However, there has been one study that found an increase in (GFAP)-positive astrocytes in a subgroup of elderly schizophrenic patients who developed dementia compared to those without dementia (Arnold et al, 1996). To date, no correlation between any of these neuropathological markers and cognitive decline in schizophrenia has been demonstrated. These findings suggest there is little histological evidence of neurodegeneration or ongoing neural pathology in schizophrenia beyond that of normal ageing thus making it more likely that the disorder is neurodevelopmental. One possible explanation for the patients who have been observed to have a deteriorating course leading to dementia, is that they may be more vulnerable to cognitive impairment with the effects of normal ageing due to abnormal neurodevelopment.

However, it is possible that the methodological differences in assessing gliosis and selecting the regions to examine may have contributed to the conflicting

neuropathological findings. More recent studies have focussed on the cortex whereas earlier studies examined the diencephalic regions. In addition, the absence of gliosis in the cortex does not exclude that possibility that it may occur in other brain structures (Jones, 1997).

v. Other evidence

Patients with schizophrenia have been reported to have increased minor physical anomalies such as reduced head circumference, low set ears or abnormal palate height (O'Callaghan et al, 1991; McNeil et al, 1993a) and soft neurological signs such as deficits in motor coordination, sensory integration or extrapyramidal abnormalities compared to healthy controls (Chen et al, 1995) suggesting abnormal central nervous system development. In addition, delays in language and social development in early childhood have been found to be more common in patients with schizophrenia compared to normal and sibling controls (McNeil et al, 1993b; Jones et al, 1994; Cannon et al, 1999). These findings lend further support to the neurodevelopmental model in schizophrenia.

Onset of pathological process

The prenatal and perinatal periods of brain development are characterised by processes of neurogenesis, neuronal proliferation, neuronal differentiation and migration. The postnatal process of synaptic proliferation continues through middle childhood and is followed by programmed elimination of synapses in adolescence when there is a refinement of brain structure and function. One theory proposes that the pathological process in schizophrenia occurs in early development. This was first suggested from epidemiological studies that reported a history of perinatal or gestational complications

in patients with schizophrenia, especially in the second half of gestation which is crucial for the development of the central nervous system in the fetus (Murray and Lewis, 1987; Weinberger, 1987). Higher rates of schizophrenia have been reported to be associated with maternal exposure to the influenza virus (Kirch, 1993) or a history of fetal malnutrition, prematurity and hypoxia (Dalman et al, 1999). Obstetric complications have also been reported to be predictive of increased brain ventricular size in adults, particularly in schizophrenia (Dalman et al, 1999). A recent case control study identified only a few prenatal and perinatal factors, namely multiparity, bleeding during pregnancy and small size for gestational age that were associated with increased risk of early onset of schizophrenia in males (Hultman et al, 1999). However, it is uncertain whether these associations are specific for schizophrenia and the underlying pathological mechanisms have not yet been established.

Further evidence that the pathological process in schizophrenia occurs in early development mainly comes from post mortem findings of cytoarchitectural abnormalities in the brain (Kovelman and Scheibel, 1984; Akbarian et al, 1993a & 1993b; Weinberger et al, 1995). Structural brain imaging and neuropathological findings (discussed in the last two chapters) suggest that an earlier timing than the middle stage of intrauterine life for the pathological process is unlikely as gross abnormalities in the structure of the cerebral cortex would be expected if neurogenesis were affected. It has therefore been proposed that the processes of programmed cell death, neural migration and synaptic proliferation which begin during the second trimester of pregnancy are most likely involved (Keshaven, 1999).

Others have suggested an alternate timing for the onset of the pathological process in schizophrenia to be later in adolescence. It has been proposed that as brain development continues throughout childhood and adolescence, the effect of damaged neurons may only become functionally apparent after cortical maturation is complete which may account for the long latency period between early cerebral insult and the onset of schizophrenia. Thus, the cytoarchitectural findings observed in schizophrenia such as abnormalities in neuronal size, synaptic and dendritic organization may originate later and may be susceptible to environmental influences or genetic factors. Reduction in membrane synthesis (Pettegrew et al, 1991), cortical grey matter volumes (Jernigan and Tallal, 1990) and prefrontal metabolism (Chugani et al, 1987) occur in normal adolescence. It has been reported that these reductions are greater in schizophrenia compared to healthy controls which suggest that there may be an exaggeration in synaptic pruning in schizophrenia in certain brain regions such as the prefrontal cortex (Keshavan et al, 1994a). This may therefore account for the synaptic abnormalities reported in these patients.

Other neuropathological processes such as abnormalities in *myelination* have also been implicated in the neurodevelopmental model of schizophrenia. Benes et al (1994) have confirmed that normal myelination continues into adolescence and adulthood, particularly in the corticolimbic areas of the brain, and therefore abnormal myelination may be relevant in schizophrenia and may account for the onset of the illness in early adulthood. Further evidence of abnormal myelination comes from recent findings from a post mortem study in schizophrenic patients of a down-regulation in the expression of myelination-related genes in the dorsolateral prefrontal cortex suggesting dysfunction in myelin forming oligodendrocytes (Hakak et al, 2001). However, only a

few neuropathological studies have examined the white matter in schizophrenia. Some MRI studies in schizophrenia (described in Chapter 2) have reported abnormalities in frontal white matter such as volume reductions (Buchanan et al, 1998; Palliere-Martinot et al, 2001) and focal signal hyperintensities (Persaud et al, 1997). In addition, white matter abnormalities have also been detected by some recent diffusion tensor imaging (DTI) studies in schizophrenia (described in Chapter 6). There is also indirect evidence from an in vivo proton magnetic spectroscopy (MRS) study that has demonstrated a reduction in the concentration of choline containing compounds, many of which are myelin components, in the hippocampi of patients with chronic schizophrenia (Maier et al, 1995). Myelin abnormalities of hippocampal connections, whether primary or secondary to axonal pathology, may also explain the clinical deterioration and progressive structural brain abnormalities reported in some longitudinal studies (Gur et al, 1998a; Rapoport et al, 1997). In addition, reports of the high incidence of psychosis in patients with metachromatic leukodystrophy, an early onset demyelinating disease (Hyde et al, 1992) lends further support to the suggestion that white matter pathology may be relevant in schizophrenia. Further studies are therefore needed to investigate the white matter in schizophrenia.

Brain connectivity in schizophrenia

It has been suggested that schizophrenia may be a disorder of brain connectivity resulting from abnormalities in the connections between different brain regions rather than within the regions themselves which may arise from neurodevelopmental defects. This theory has mainly originated from functional imaging studies that have suggested that schizophrenia can be understood as failure of functional integration of the brain in cognitive terms and pathophysiology, particularly involving the frontal and temporal

lobes. It has been observed that there are clinical similarities between patients with schizophrenia and patients with organic psychoses such as temporal lobe epilepsy, tumours, trauma, infections and neurodegenerative diseases such as Alzheimer's or Pick's, that selectively affect the temporal and frontal lobes as well as the basal ganglia. The evidence of disrupted connectivity in schizophrenia has been provided by the following:

i. Functional imaging studies

Functional imaging studies have permitted the exploration of relationships between schizophrenic symptomatology and cognitive tasks with brain function. It has been postulated that a disruption in the integration of functionally specialised systems for adaptive sensorimotor integration and cognition occurs in schizophrenia which may or may not have macroscopic anatomical correlates (Friston, 1999). Earlier positron emission tomography (PET) studies reported reduced cerebral blood flow in the frontal cortex or 'hypofrontality' which was associated with prominent negative symptoms (Ingvar et al, 1974; Volkow et al, 1987).

The findings from current functional imaging studies in schizophrenia suggest that schizophrenic symptomatology is associated with a disruption in distributed functional circuits rather than an abnormality in a single brain region, predominantly involving the frontal and temporal lobes, although other brain regions such thalamus, anterior cingulate and cerebellum have also been implicated. In particular, some studies have reported increased cerebral blood flow in left mesiotemporal structures, right anterior cingulate, left superior temporal and dorsomedial thalamus to be correlated with positive symptoms such as delusions and hallucinations (Liddle et al, 1992; Friston et

al, 1992) whereas decreased in cerebral blood flow was detected in the prefrontal and parietal cortex in patients with negative symptoms such as psychomotor poverty (Liddle et al, 1992; Tamminga et al, 1992). Another PET study in patients who experience auditory verbal hallucinations reported a failure to activate areas implicated in monitoring of inner speech, namely the left middle temporal regions and supplementary motor areas in these patients (McGuire et al, 1996b).

ii. Neuropsychological studies

A range of neuropsychological deficits have been reported in patients with chronic schizophrenia (McKenna et al, 1990; Crawford et al, 1993; Mellers et al, 2000) and first episode patients (Saykin et al, 1994; Hoff et al, 1992; Hutton et al, 1998; Gur et al, 1998a), particularly affecting attention, memory and executive functions. Apart from the structural imaging studies described in Chapter 2 that have correlated fronto-temporal brain abnormalities with the neuropsychological deficits observed in schizophrenia, further support comes from functional imaging studies, particularly using PET.

Cognitive activation studies in PET require the subject to perform a cognitive task, such as verbal fluency, whilst being scanned. These studies have demonstrated the involvement of frontal and temporal lobes in schizophrenia either in isolation or being inter-related. Reduced frontal metabolism during a frontal task has been observed in schizophrenic patients (Buchsbaum et al, 1992; Taylor et al, 1996). There is some evidence that these changes are unlikely to be related to long term use of neuroleptic medications from a study that reported decreased blood flow in left mesial frontal region on single photon emission computed tomography (SPECT) in both chronic

schizophrenic patients who were medication free for at least 3 weeks and drug naïve patients when they were compared to controls during their performance on the Tower of London task (Andreasen et al, 1992). Weinberger et al (1992) found an association between left hippocampal volume reduction and decreased cerebral blood flow in the dorsolateral prefrontal cortex during the Wisconsin Card sorting test in the affected twin of monozygotic twins discordant for schizophrenia. Others have reported a failure of deactivation in the temporal lobes normally associated with activation of dorsolateral prefrontal cortex during a verbal fluency task in schizophrenic patients (Frith et al, 1995; McGuire and Frith, 1996a). Further support for impaired fronto-temporal connectivity in schizophrenia comes from recent studies using fMRI which have demonstrated reduced activation in the frontal and temporal regions during verbal memory and executive tasks (Yurgelun-Todd et al, 1996b; Ragland et al, 1998).

Some researchers have proposed that a more complex disruption of distributed neural circuits is involved in schizophrenia. Andreasen et al (1996) have postulated that the symptoms of schizophrenia arise from impaired connectivity between frontal, thalamic and cerebellar regions which has been supported by PET findings of reduced perfusion in prefrontal, inferior temporal and parietal cortex with increased perfusion in the thalamus and cerebellum in a group of unmedicated or drug naive schizophrenics during their performance on practised and novel recall tasks (Andreasen et al, 1996 & 1997). It has been suggested that this disruption in the corticothalamocerebellar circuits results in 'cognitive dysmetria' which is defined as a failure in coordinating and monitoring the process and retrieval of cognitive information. It is postulated that the prefrontal regions serve executive functions which involve prioritising data and placing it within a broad contextual meaning, formulating decisions and initiating

action; the thalamus serves as a filter by receiving sensory information and forwarding the relevant information while the cerebellum coordinates and processes the information from cortical and subcortical regions.

iii. Structural imaging studies

Less is known about the anatomical connections between different regions in schizophrenia and very few structural imaging studies have investigated this. Breier et al (1992) reported correlations between prefrontal and temporal lobe volumes and between right prefrontal white matter volume and right amygdala-hippocampal volumes suggesting abnormal limbic-cortical connections in schizophrenia. Woodruff et al (1997) has reported a dissociation between frontal and temporal lobe volumes in schizophrenic patients compared to controls which lends further support to the suggestion that fronto-temporal connections are disrupted in schizophrenia. Further evidence comes from more recent studies that have reported supra-regional changes within the fronto-temporal system in schizophrenic patients using 3D voxel-based analyses (Wright et al, 1999; Sigmundsson et al, 2001). Another study reported abnormal correlations between thalamic and prefrontal white matter volumes in a group of chronic male schizophrenic patients suggesting that thalamocortical connections may also be disrupted in schizophrenia (Portas et al, 1998). These studies suggest that the pathological changes in schizophrenia involve the disruption of neural circuits in anatomically interconnected brain regions, particularly the fronto-temporal or prefrontal-limbic ones.

iv. Neuropathological/ histopathological studies

The histopathological findings in schizophrenia mainly involve the frontal and temporal regions and have been described in Chapter 3. Although some of these findings have been conflicting, such as the reports of neuronal abnormalities in schizophrenia, others have been more consistent. Reports of dendritic and synaptic abnormalities in the frontal and temporal regions have led to the suggestion the underlying pathophysiological mechanism of altered connectivity in schizophrenia may be at a microscopic or cellular level. Further evidence of a disruption of fronto-temporal connections in schizophrenia comes from the few post mortem investigations that have reported alterations in GABA and glutamate in the frontal and temporal brain regions in schizophrenia which may reflect the disruption of connecting pathways such as corticostriatal, thalamocortical and corticocortical association fibres in schizophrenia. It is evident that further investigations are needed to confirm these findings.

CHAPTER 5

MAGNETIZATION TRANSFER IMAGING

Since the 1970s, magnetic resonance imaging (MRI) has developed into one of the most important diagnostic tools. MRI is a non invasive, safe and sensitive tool that does not use radiation to investigate the microstructure of soft tissues and has been used widely to investigate brain disorders. Structural brain abnormalities detected on conventional magnetic resonance imaging (MRI) are gross by definition with loss of volume or focal lesions indicating an obvious pathological process. More subtle abnormalities, which may nevertheless have functional significance, are more difficult to detect by conventional MRI. Furthermore, conventional MRI also lacks pathological specificity as a range of disease processes such as oedema, inflammation or gliosis may result in a similar signal change. This has led to the search for MRI techniques, such as magnetization transfer imaging (MTI) or diffusion tensor imaging (DTI), which have the potential for providing more specific neuropathological information in vivo and may be more sensitive to subtle or early neuropathological changes that may be undetectable by conventional MRI.

The principles of conventional MRI have been well described (Andrew et al, 1990) and will only be discussed here as far as they are relevant to an understanding of MTI. Measured MRI signal characteristics depend on the physical and chemical processes experienced by water molecules in tissues, predominantly by the contribution of freely mobile water protons because of their great abundance and sharp resonance frequency. Water in tissue exists in a 'bound' state and a 'free' state, and water molecules are

exchanged freely between the two states, with the exchange rate being a function of the tissue type. This is illustrated in Figure 1.

Water molecules remain bound only for a short time, after which they become unbound. In conventional MRI sequences, the contrast on MR images results from the differences in relaxation properties and proton densities of the free mobile water protons. However, there also exists a separate pool of water protons which are tightly bound to macromolecular structures such as proteins and cellular membranes. Free water protons are fast moving and have relatively long T2 relaxation times (approximately 50milliseconds) which gives rise to the MR signal on conventional MRI. Bound water protons are slow moving, have very short T2 relaxation times (less than 100 microseconds) and are therefore essentially not detected on conventional MRI. The free pool has a discrete and a sharp central peak on MR spectroscopy (approximately 20Hz) whereas the bound pool has a wide spectrum centred symmetrically around the free peak (greater than 10 Hz). This is illustrated in Figure 2.

Figure 1. Bound and free water

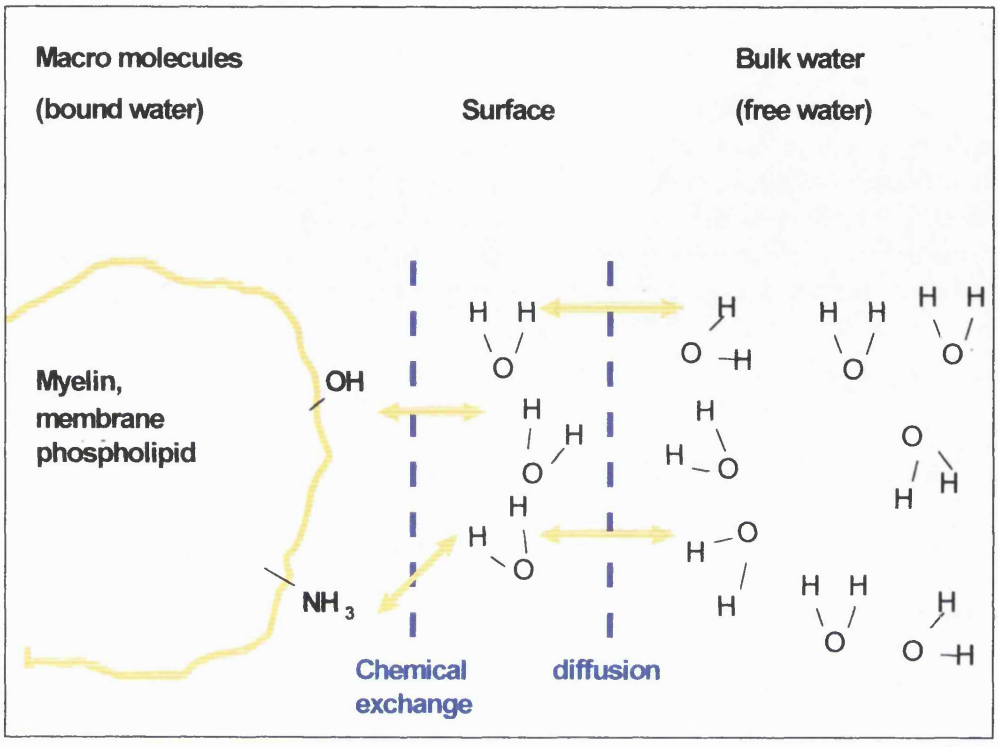
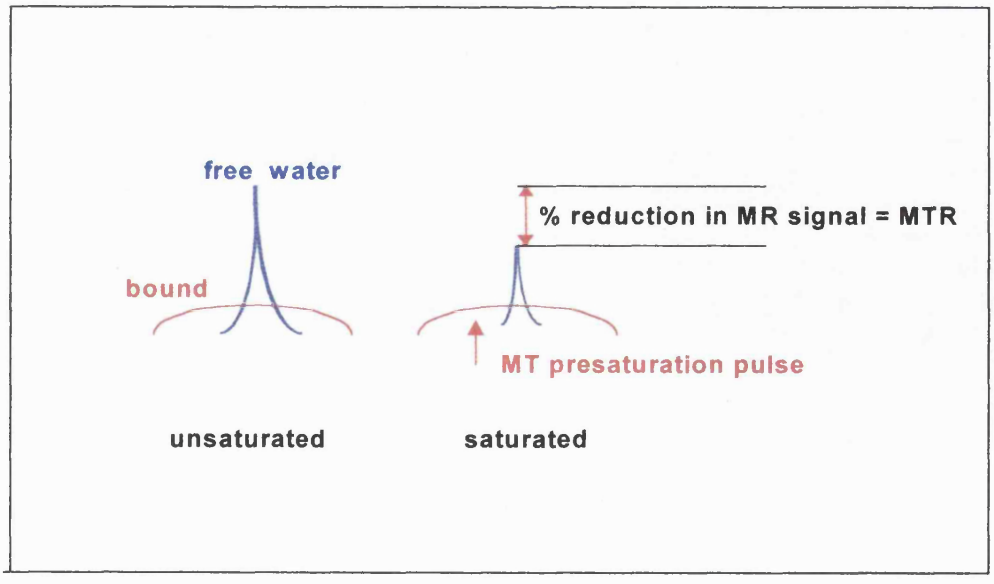


Figure 2. Magnetization transfer



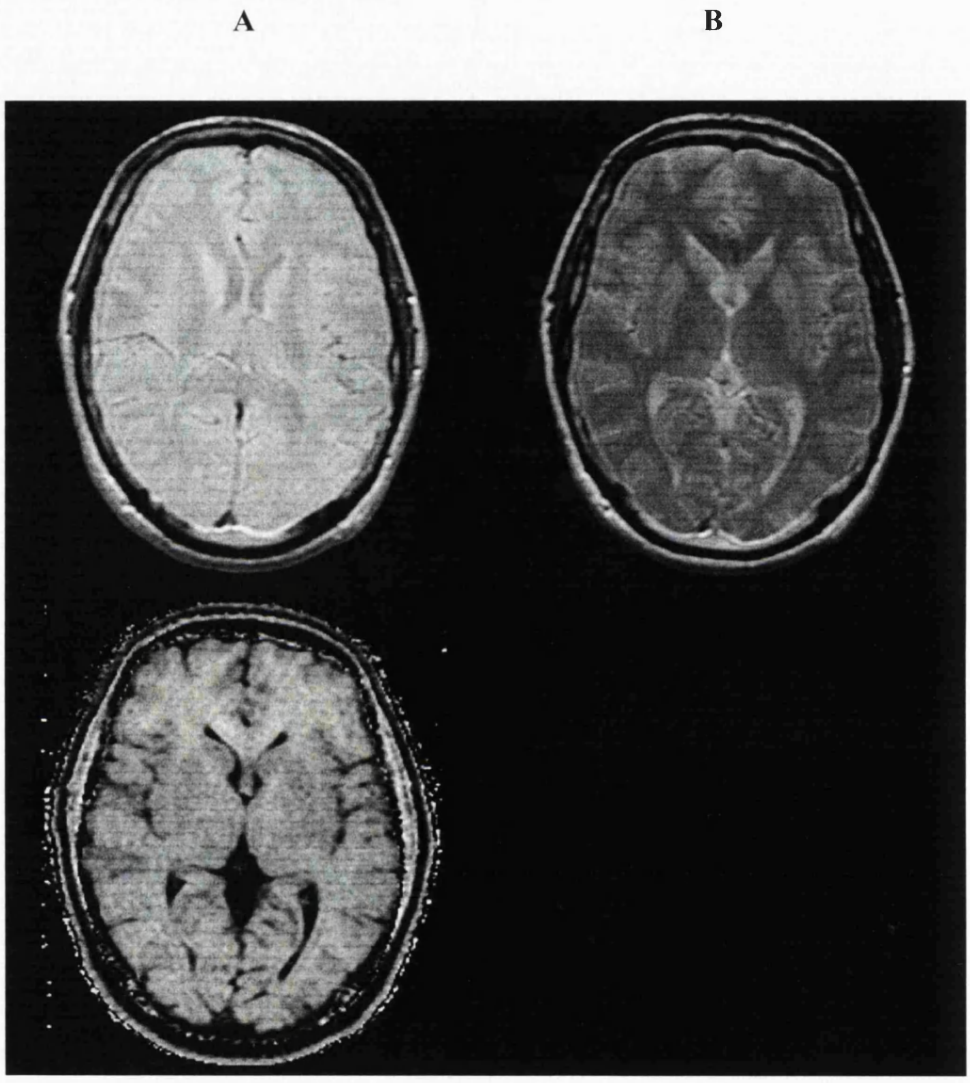
Magnetization transfer imaging (MTI) allows the indirect visualisation of these bound protons which are essentially invisible to conventional MRI sequences due to their very short T2 relaxation times. The bound protons have restricted motion and interact with mobile water protons through chemical exchange. Magnetization transfer techniques use an off resonance radio frequency (RF) pulse that is centred well away (about 1kHz) from the water frequency to selectively saturate protons bound to macromolecules without affecting the free water pool directly (Figure 2). The exchange of magnetization leads to a reduction in the signal of the free pool usually of the magnitude of 50-60%, resulting in a decrease in tissue signal intensity on an MR image. Thus, in a MTI sequence, two sets of images are obtained, the unsaturated and saturated images, and MT images are produced by digital subtraction as shown in Figure 3.

The percentage reduction in MR signal is known as **Magnetization Transfer Ratio** (MTR) and directly reflects the amount and nature of macromolecules in a given volume (Wolff and Balaban, 1989). Thus, MTR provides a quantitative measure of macromolecular structural integrity and can be calculated on a pixel by pixel basis from the formula:

$$MTR = \{[M_0-M_S]/M_0\} \times 100$$

where M_S and M_0 are the mean signal intensities determined for a given region with and without the saturation pulse respectively.

Figure 3. Spin echo MTR sequence



A = unsaturated; B = saturated; C = MTR image

MTI was first used to enhance tissue contrast on MR images. This was termed magnetization transfer contrast by Wolff and Balaban (1989) following observations that it was possible to generate good tissue contrast using this technique particularly for imaging the brain. Strong magnetization transfer saturation effects are observed in tissues with strong protein or macromolecular matrix such as skeletal/cardiac muscle and brain. In these cases, when a long MT pulse is applied prior to the imaging pulse, the above mentioned tissues will be suppressed. Tissues that exhibit MT effect in reducing order of magnitude are myelinated white matter, grey matter, muscle, blood and CSF. Therefore different MTR values are observed in different tissues in the normal brain with the highest values in white matter (30-50%), medium in grey matter (20-40%), very low in CSF (~0%). Low MTR would indicate a reduced capacity of the molecules in the brain tissue matrix to exchanges magnetization with the surrounding MR-visible water molecules.

The analysis of data from MTI has been under development in the past few years. The techniques that have been previously used include a region of interest (ROI) analysis and histogram analysis. In a ROI approach, regions of a predetermined shape or anatomical configuration can be manually placed over a structure of interest by the investigator and in an automated way, quantitative values for both MTR measures can be obtained from MT images. However, they are subject to observer bias and partial volume effects must be considered. In order to minimise observer bias, the ROIs can be defined on a coregistered image, such as T2 or proton density, and not directly on the image map to be studied. It is also possible to map the contours around lesions and to obtain MTR values in the lesions or in adjacent areas. This has been demonstrated by Filippi et al (1995) who reported that MTR is reduced in normal appearing white

matter adjacent to multiple sclerosis (MS) lesions indicating that the boundaries of the lesions are irregular and extend into normal appearing white matter which does not occur in other lesions such as diffuse axonal injury.

Another technique that has been used to analyse MTI data is the volumetric technique of histogram analysis (von Buchem, 1996). This technique has mainly been applied to MTR data from studies in MS. The histograms are based on calculated MTR and several parameters can be derived including the height, position of the histogram peak and the average MTR. The single sharp peak corresponds, in the normal brain, to normal white matter pixels. Brain MTR histogram measures are stable over time in healthy controls as demonstrated in a one year longitudinal study (Filippi et al, 2000b). However, there is some variability with the use of different MR scanners and it may therefore be difficult to compare results between different studies using this method of analysis. Global histogram analysis offers a method for quantifying overall disease burden and is likely to prove useful for monitoring disease progression or in therapeutic trials.

Reproducibility

MTR measurements have been shown to be reproducible over time in phantom and in vivo measurements in normal control subjects (Barker et al, 1996). Barker et al (1996) developed an interleaved dual spin echo based sequence in which inherently registered MT and non-MT images are produced. They demonstrated that repeat scans in a control subject in the same scanner showed relatively little change over a short time (one week) and was also stable with repositioning reflecting the scanner stability and precision of the prescan setup. They also studied a group of normal controls and

reported that MTR measurements in different regions of the brain were consistent between subjects. High intra and inter rater reliability of MTR measurements in healthy controls has also been demonstrated in a recent study (Sormani et al, 2000).

An essential consideration is that MTI techniques need to be standardised to allow comparison between the numerical values of MTR across the different studies. MTR is dependent on the sequences and spatial uniformity of head coils for saturation and it is known that different centres use different MT techniques which makes it difficult to directly compare MTR results between different studies. Therefore, results from studies using MTI must be carefully interpreted.

Age and gender effects

Subtle age related changes have been observed in healthy controls (Silver et al, 1997, Traboulsee et al, 2001). Silver et al (1997) examined MTR measures in white matter for 41 subjects using a region of interest approach. They compared two age groups (16-35 and 36-55 years) and found that increasing age was associated with a reduction in MTR in white matter, particularly in the corpus callosum and frontal regions. In this study, no gender differences in MTR were demonstrated which supported previous findings (Mehta et al, 1995).

A more recent study (Traboulsee et al, 2001) examined MTR using histogram analysis in a larger sample of 90 healthy controls aged 18-56 years. In contrast to the findings of Silver et al (1997), there were no age related changes in white matter. However, age related MTR changes in grey matter were detected as reflected in the histogram peak height whilst average MTR remains stable. In addition, there were gender differences

with higher average MTR for whole brain and grey matter and higher white matter peak height in women compared to men.

Regional variations

Regional differences in MTR in white matter have been observed with the corpus callosum having the highest MTR values reflecting the high density of myelinated white matter fibres (Silver et al, 1997; Mehta et al, 1995) and higher MTR in frontal white matter compared to other regions such as the parieto-occipital white matter (Silver et al, 1997). In addition, interhemispheric differences with higher MTR values in the left hemisphere compared to the right which were not related to handedness have also been reported (Silver et al, 1997). It has been proposed that this may reflect differences in fibre packing density or myelination. In contrast to the findings in white matter, no regional differences in MTR have been reported in grey matter (Mehta et al, 1995).

Application in neurological disorders

The two main areas of clinical application of MTI are in contrast enhancement and in tissue characterization. One of the first clinical applications of contrast augmentation was in MR angiography. Blood, being a semifluid, has a lower rate of MT than other tissues. This leads to enhanced contrast between tissue and blood on MR images which therefore improves the delineation of blood vessels in the brain and heart. MT contrast can also be combined with a paramagnetic contrast agent such as gadolinium-diethylene triaminopentaacetic acid (DTPA). In conventional MRI, gadolinium-DTPA, provides better image contrast for tissue differentiation. Blood vessels or lesions in the brain which enhance with gadolinium-DTPA on T1-weighted images are more visible

with the addition of MTI because MTI suppresses normal tissue so that areas of enhancement are more conspicuous. The combination of MT imaging with gadolinium-DTPA has been shown to increase the sensitivity in detecting contrast enhancing lesions in MS (Silver et al, 1997). Other studies (Han et al, 1998; Haba et al, 2001) have demonstrated that brain tumours, particularly, meningiomas, enhance as effectively with half dose gadolinium-DTPA and MT imaging as the use of standard dose gadolinium-DTPA alone.

MTI can be more useful than conventional MRI in tissue characterization and has been used to investigate a number of neurological diseases described below. The range of MTR in grey matter is lower than in white matter which may reflect the greater density of myelinated fibres in white matter. Abnormalities of MTR in grey matter, however, have not been studied as extensively as in white matter and therefore their neuropathological correlates remain to be determined.

Demyelination/Multiple sclerosis

It was first demonstrated in animal studies that MTR was slightly reduced with oedema and more greatly reduced in demyelination and axonal loss in experimental allergic encephalomyelitis (Dousset et al, 1992). Large reductions in MTR have been reported in neurological conditions where there is significant myelin loss such as multiple sclerosis (Gass et al, 1994; Thorpe et al, 1995), progressive multifocal leukoencephalopathy (Dousset et al, 1997) and central pontine myelinolysis (Silver et al 1996). Post mortem studies in multiple sclerosis have reported significant correlations between MTR values in MS lesions and axonal density (Mottershead et al, 1998; van Waesberghe et al, 1998) which indicate that MTR abnormalities in white

matter may also be related to axonal loss. MTR also allows the subcategorization of multiple sclerosis lesions with reports of greater reductions in chronic lesions reflecting demyelination compared to smaller reductions in acute lesions related to oedema (Werring et al, 1998). In cases of wallerian degeneration (degeneration in the distal axon and its myelin sheath secondary to proximal axonal injury), use of MTR appears to allow reliable detection of changes undetectable with conventional MRI (Grossman et al, 1994).

Importantly, MTR appears to be sensitive to subtle changes in lesions or in normal appearing brain tissue. Most of the evidence that MTR is able to detect abnormalities in normal appearing brain tissue that are undetected on conventional MRI arises from studies in MS. It has been found that MTR values are reduced in the normal appearing white matter (NAWM) surrounding MS lesions and progressively increase when moving further away from the centre of MS lesions (Filippi et al, 1995). This is in contrast to traumatic brain injury where there is an abrupt transition in MTR values between the lesions and NAWM (Bagley et al, 1999). More recently, it has been demonstrated that MTR in normal appearing grey matter (NAGM) was decreased in patients with multiple sclerosis compared to controls suggesting the presence of subtle abnormalities in grey matter such as small MS lesions or wallerian degeneration of grey matter neurons that are not detected on conventional MRI (Cercignani et al, 2001a). Another study reported reduction in MTR in grey matter in a group of relapsing remitting MS patients compared to controls which correlated significantly with clinical disability suggesting that MS is a more diffuse disease affecting the whole brain (Ge et al, 2001). MTR reductions in lesions (Rovaris et al, 1998), NAWM (Rovaris et al, 1998) and NAWM together with NAGM (Filippi et al, 2000c) have

been reported to correlate significantly with neuropsychological deficits and may therefore reflect the true extent of brain disease more accurately that measuring T2 lesion load on conventional MRI.

All dimensions of MS pathology including changes in both acute and chronic lesions, microscopic changes in normal appearing tissue and atrophy can be reflected by MTR histogram analysis. The peak height and average MTR on histogram analysis are significantly reduced in patients with multiple sclerosis compared to healthy controls. MTR histograms has also been found to provide information about the level of impairment and disability in patients with a 'relapse onset' and therefore is valuable in monitoring the evolution of disease in these patients (Kalkers et al, 2001). It has also recently been suggested that MTR may be used to monitor the efficacy of MS therapies in promoting myelin repair from a report that spontaneous myelin repair following toxic demyelination in an animal model was significantly associated with reduced MTR values returning to normal (Deloire-Grassin et al, 2000). In addition, reductions in average MTR on histogram analysis have been demonstrated in secondary progressive MS patients only after a year indicating that this technique is sensitive in detecting short term changes in MS pathology and may therefore be useful in monitoring clinical trials (Filippi et al, 2000b).

Leber's hereditary optic neuropathy, a neurological condition that is considered to be associated with multiple sclerosis has also been studied using MTI (Inglese et al, 2001). Apart from lower MTR in the optic nerve in patients compared to controls, the MTR peak height on histogram analysis was also reduced in normal appearing brain tissue reflecting microscopic pathology.

Tumour

MTI has also been applied to study brain tumours which have been reported to have reduced MTR compared to normal brain tissue. MTI also appears to be better at tissue characterization of some intracranial tumours than conventional MRI. It has been shown to be superior to T2 contrast in the discrimination of low grade astrocytomas, haemangiolastomas and craniopharyngioblastomas, which were found to have lower MT ratios than other brain tumours such as meningiomas, pituitary adenomas and acoustic neuromas (Kurki et al, 1995; Okumura et al, 1999). In these studies, MTI was able to differentiate between low and high grade astrocytomas as low grade astrocytomas had significantly lower MTR than the high grade ones. However, there were limitations in that MTI could not discriminate between meningiomas, high grade astrocytomas and metastases.

Vascular

There is some suggestion that MTR may be sensitive in detecting ischaemic damage in the brain. A recent study has examined a small sample of patients with unilateral internal carotid artery stenosis and demonstrated that the reduction in MTR in NAWM was significantly correlated with regional cerebral metabolic rate of oxygen observed on PET (Kado et al, 2001). These findings indicate the potential use of MTI in assessing ischaemic brain injury.

In a small study, patients with systemic lupus erythematosus (SLE) who had a previous history of neuropsychiatric complications were found to have global reductions of MTR when compared to those without a history of neuropsychiatric complications (Bosma et al, 2000). The findings suggest that MTI is capable of

detecting CNS damage in these patients which is undetected on conventional MRI and may be useful in studying the natural history of the disease.

Trauma

Detection of acute axonal damage in traumatic brain injury using MTR which was undetected by conventional MRI was first demonstrated in animal studies. MTR has been found to correlate with diffuse axonal injury resulting from traumatic brain injury in pigs (Kimura et al, 1996). The potential for extending this to humans may be increasingly important given the recent suggestions that damage to axons and dendrites in traumatic brain injury may be dramatically reduced in the acute phase by pharmacological intervention. A recent study in a small sample has demonstrated that MTR abnormalities can be detected in NAWM three months after traumatic brain injury suggesting that MTR could potentially be used to quantify axonal damage following this kind of injury (Sinson et al, 2001).

Intracranial infection

MTI has also been used to study white matter pathology in patients with acquired immunodeficiency syndrome (AIDS) and has been shown to be able to differentiate between progressive multifocal leucoencephalopathy (PML) and non specific human immunodeficiency virus (HIV)-associated white matter lesions (Ernst et al, 1999). Significantly greater MTR reductions were found in PML than in HIV-associated white matter lesions which reflects the different pathophysiological mechanisms. PML is caused by infection of oligodendrocytes by the JC virus resulting in demyelinating lesions whereas HIV associated white matter lesions are primarily related to gliosis.

These findings also suggest that longitudinal studies using MTR may be helpful in monitoring disease progression and therapy in patients with PML.

Application in psychiatric disorders

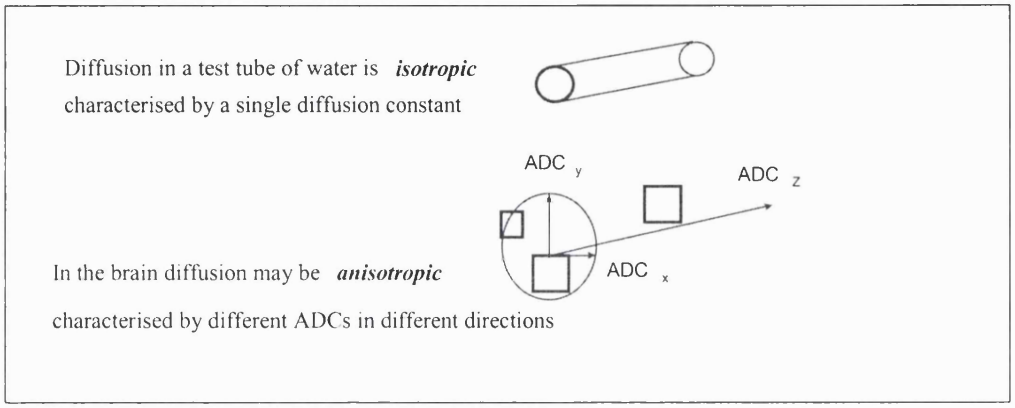
MTI has not been previously applied to psychiatric disorders. The work in this thesis with MTI is the first time this technique has been used to investigate schizophrenia.

CHAPTER 6

DIFFUSION TENSOR IMAGING

All molecules in liquid or gases undergo random (Brownian) motion as a result of interacting with other molecules. The distances travelled during diffusional motion in a given time are characterised by the self diffusion coefficient (Horsfield et al, 1998). In biological tissues, diffusion is not truly random because of partially permeable tissue structures (e.g. cell membranes, vasculature and axon cylinders) which restrict the amount of diffusion. Thus, the diffusion coefficient measured in vivo by MRI is always lower than that of free water and is referred to as 'apparent diffusion coefficient' (ADC) (Le Bihan et al, 1998). Isotropic diffusion occurs when the diffusion properties of the water molecules are equal in all directions. For cellular structures like axonal fibres, diffusion will be much less restricted when the motion is along rather than across the fibres. Diffusion that has a strong directional component is known as anisotropic (Hajnal et al, 1991) and can be characterised by different ADCs in different directions (Figure 4).

Figure 4. Isotropic and anisotropic diffusion



Diffusion Weighted Imaging (DWI), a non-invasive MRI technique, is capable of measuring diffusion of water in the central nervous system. Water molecules diffusing in and around axons are affected by the presence of cellular structures such as cell membranes that provide barriers which hinder their motion (Hazelwood et al, 1991) and thus the water molecules undergo restricted diffusion. DWI therefore provides information about the size, orientation and shape of cellular brain structures in vivo.

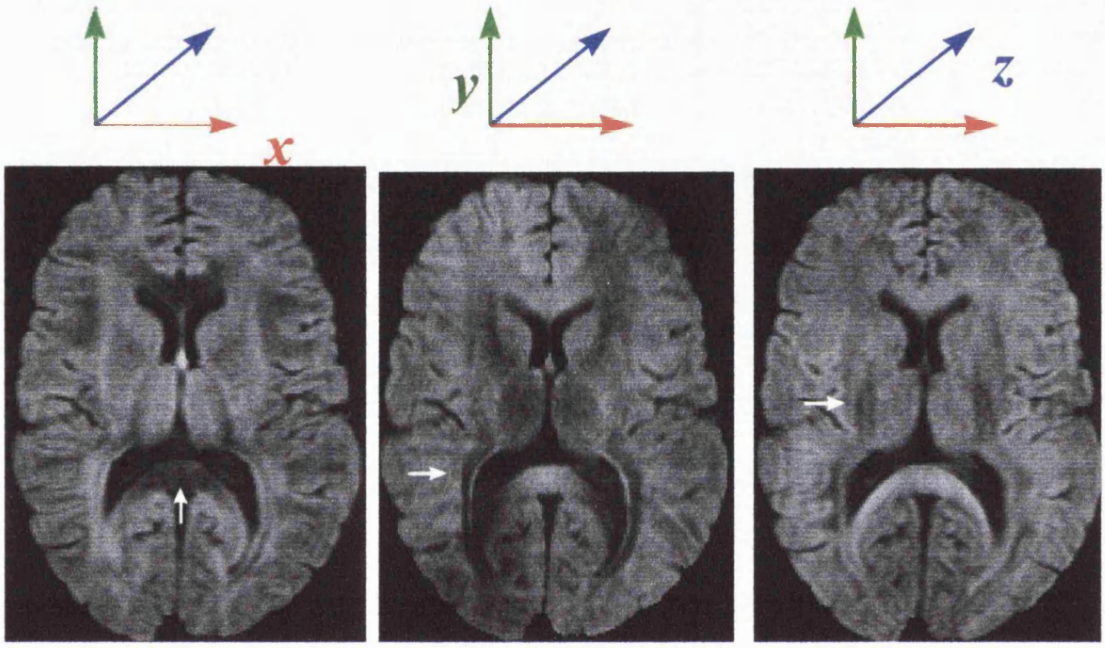
In the presence of a magnetic field gradient on MRI, spins accumulate different phase shifts due to their diffusional motion, resulting in reduced MR signal intensity but these effects are very small. The sensitivity to diffusional motion can be increased by adding strong magnetic field gradient pulses. DWI is therefore not usually available on standard MR scanners due to the powerful field gradients required. DWI is performed optimally on a high-field (1.5 Tesla) scanner and using an ultrafast imaging mode such as echo planar imaging (EPI). EPI will enhance the signal as DWI has a low signal to noise ratio and also reduce motion artefacts from head motion or pulsatile motion of the CSF. It also allows fast data collection as multiple independent acquisitions can be averaged without long scanning times.

To obtain diffusion-weighted images, a pair of strong gradient pulses are added to the pulse sequence. The first gradient pulse dephases the spins, and the second pulse rephases the spins if no net movement occurs. If net movement of spins occurs between the gradient pulses, signal attenuation occurs. The degree of attenuation depends on the magnitude of molecular translation and diffusion weighting. The amount of diffusion weighting is determined by the strength of the diffusion gradients, the duration of the gradients, and the time between the gradient pulses.

The signal intensity on diffusion-weighted images depends on the spin density, T1, T2, TR, and TE. To eliminate these influences and obtain pure diffusion information, diffusion coefficient maps can be calculated. A diffusion map can be calculated by combining at least two diffusion-weighted images that are differently sensitised to diffusion but remain identical with respect to the other parameters, spin density, T1, T2, TR, and TE (e.g. using an image without diffusion weighting [b=0] and one diffusion-weighted image [$b > 0$]). Diffusion data can be presented as an image map of the ADC.

Alterations in ADC occur with changes in either the intracellular and extracellular compartments induced by local physiological imbalances, such as those caused by local ischaemic conditions. Thus, ADC increases when there is damage to diffusion barriers and decreases with cell swelling (cytotoxic oedema) which occurs in cerebral ischaemia. Tissues with high ADC such as CSF, produce low signal intensity and therefore appear dark on images whereas those with low ADC produce high signal intensity on images. In a fibre tract, diffusion of water is much greater along than across the fibre and DWI can therefore reveal some information about the directionality of the tracts. Images acquired using a navigated spin echo diffusion weighted sequence with the diffusion gradients applied in three different directions (x,y,z) are shown in Figure 5.

Figure 5. Navigated spin echo DWI (control subject)



a) **diffusion gradients left to right; low signal in corpus callosum**

b) **diffusion gradients vertical; low signal in frontal white matter tracts and optic radiation**

c) **diffusion gradients perpendicular; low signal in corticospinal tracts**

MRI can only measure diffusion along one direction at a time and therefore different directionally dependent components must be measured separately. Diffusion inside three dimensional structures cannot be described by a single diffusion coefficient and a mathematical entity known as the diffusion tensor is needed to fully characterise the diffusion (Basser et al, 1994). This a 3 x 3 matrix of numbers that describes diffusion in three dimensions. Thus, diffusion tensor is represented by:

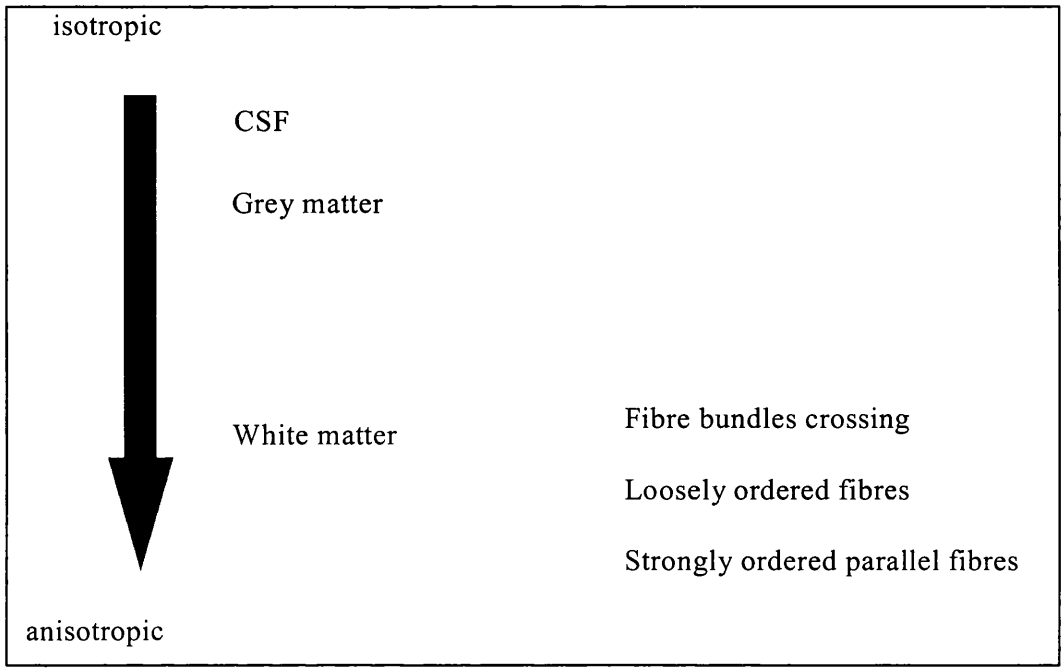
$$\mathbf{D} = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix}$$

This more recently developed and applied technique is known as **Diffusion Tensor Imaging (DTI)** in which diffusion weighted gradients are applied in at least six non-collinear directions, at a number of gradient strengths. Diffusion information can therefore be accessed in the three dimensions by using several separate images formed with the diffusion encoding gradient pulses applied independently in a different direction for each image. This means that diffusion data sets contain many images but the use of EPI shortens the acquisition time. The tensor can be diagonalised to give three eigenvectors representing the principle directions of diffusion (x,y,z) and three eigenvalues (e1, e2, e3) representing the magnitude of diffusion along these directions. The on-diagonal elements of the tensor represent the diffusion coefficients along the axes of the reference frame, while the off-diagonal elements account for the correlation existing between orthogonal directions.

It is possible to derive some measures that reflect the diffusion characteristics of the tissue from the tensor. The trace of the tensor, which is equal to the sum of the on-

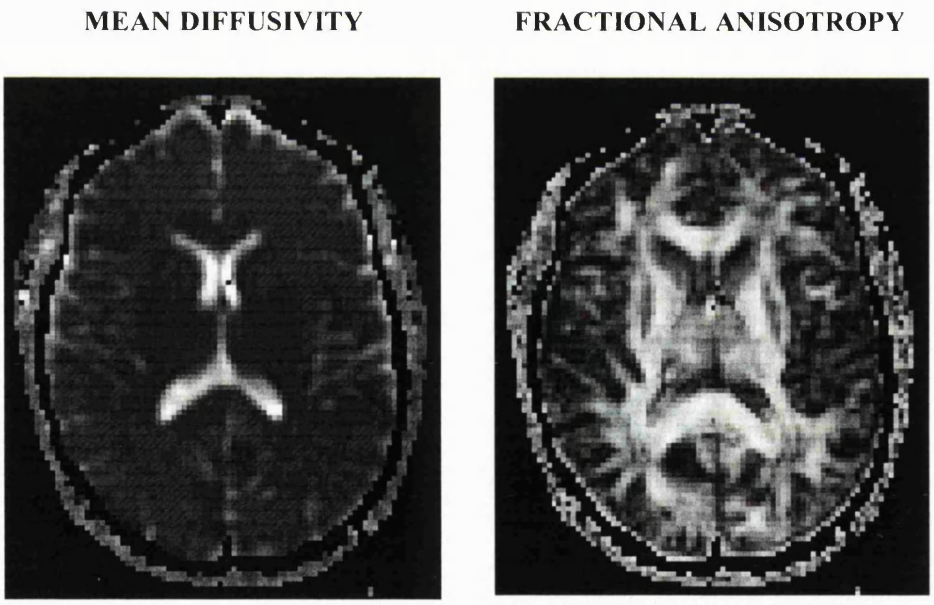
diagonal elements (e1+e2+e3), is a rotationally invariant measure of diffusion which means that for any three orthogonal directions, the sum of the measured diffusion rates will be equal within a given voxel. One third of the trace is referred to as **mean diffusivity (D)**, which is the average diffusion coefficient that can be obtained by averaging the ADC measured in three orthogonal directions without estimating the full tensor. Another measure that can also be derived from the tensor is **fractional anisotropy (FA)** which is an index of the deviation from isotropy and also reflects the alignment and integrity of cellular structures within fibre tracts. Thus, anisotropy is lower in grey matter and CSF than in white matter due to the reduction or absence of fibre tracts. The range of anisotropy within white matter is variable, with the greatest values in highly ordered parallel fibre structures such as the corpus callosum and lower values in regions where fibres have different orientations or where fibre bundles cross (Figure 6).

Figure 6. Diffusion in the brain



It is also possible to examine the intrinsic fibre directions for each voxel and to display the directionality of diffusion on colour coded maps and more recently, fibre tractography has been used to reconstruct fibre tracts (Basser et al, 2000). Thus, DTI provides quantitative measures of averaged diffusion (mean diffusivity) and diffusion anisotropy (fractional anisotropy) which can be calculated from the diffusion maps as shown in Figure 7.

Figure 7. Diffusion maps



Post mortem correlations with pathology for diffusion parameters have not been studied. Therefore, the precise histopathological correlates of DWI changes have not been determined. However, it is apparent that DWI is very sensitive to the direction of the axons in white matter. The measured diffusion coefficients appear to be larger when measured along the fibres (in the range of 1.0×10^{-3} mm^2/sec) than across the fibres (in the range of 0.6×10^{-3} mm^2/sec) which results in anisotropic diffusion (Chernervert et al, 1990; Pierpaoli et al, 1996). Thus, it is possible to study the orientation and organisation of fibres in white matter tracts using DWI which are not discernible with conventional MRI. Myelin sheaths around axons, the axonal membrane and neurofibrils (neurofilaments, microtubules) are the structural components in white matter that impede water mobility and give rise to anisotropy (Beaulieu et al, 1994). DWI has been able to provide early detection of brain myelination which is completed by age of six months compared to conventional MRI (Nomura et al, 1994). However, more recent research has indicated that myelin is not the only determinant and other structural features of axons also contribute. In fact, a recent study (Miranda et al, 1998) demonstrated similar fractional anisotropy values in partially myelinated or unmyelinated white matter structures in the infant brain compared to the fully myelinated adult brain indicating that the contribution of myelin abnormalities to changes in anisotropy may be less significant than that from axonal abnormalities. Reduced fractional anisotropy may therefore reflect a disruption in the organisation of tracts (Werring et al, 1998; Wieshmann et al, 1998) and has been reported in a range of structural cerebral abnormalities of different aetiologies such as brain damage (postsurgical, perinatal, traumatic), dysgenesis (cortical dysplasia, heterotopias) and tumours (meningioma, glioma) (Wieshmann et al, 1999).

Application in neurological disorders

Earlier studies used DWI sequences but more recent ones have used the newer DTI sequences in the investigation of neurological disorders. To avoid confusion in terminology, DWI will be referred to in the studies described below but it will be specified where the newer DTI sequences have been applied.

Vascular

DWI has proven to be effective in the early detection of strokes. Early (within the first 6 hours after stroke) signs of brain ischaemia are subtle and can be difficult to detect on conventional MR images. The early morphologic signs produced by tissue swelling are detected in about 50% of acute infarctions but signal abnormalities on conventional MRI are not seen. However, DWI is very sensitive and specific in detecting acute infarction and most infarcts can be detected as early as 30 minutes after an acute stroke event and diffusion remains significantly reduced for 3 to 5 days (Schwamm et al, 1998). This is helpful in the differential diagnosis of stroke as normal DWI in these patients may suggest other diagnoses such as transient ischaemic attacks, migraines, seizures or peripheral vertigo (Lovblad et al, 1998).

In acute cerebral ischaemia where there is a reduction in cerebral blood flow, the cell membrane ion pump fails and excess sodium enters the cell, which is followed by movement of water from the extracellular to intracellular compartment. This causes the cells to swell, a condition commonly known as cellular or cytotoxic oedema. Diffusion of the intracellular water molecules is restricted by the cell membranes. The restricted diffusion results in a decreased ADC and in severe ischaemia, ADC can be reduced by as much as 56% of normal tissue at 6 hours. This appears as an area of increased signal

on a diffusion-weighted MR image. It has been suggested that DWI could be used in detecting non-symptomatic acute ischaemic events before and after carotid endarterectomy (Tomczak et al, 2001).

DWI also helps to differentiate acute from chronic lacunar infarctions. As people age, they commonly develop lesions in the brain related to longstanding small vessel ischaemia which can be difficult to differentiate from more acute ischaemia. Acute infarcts appear hyperintense on the diffusion images whereas chronic infarcts or changes of deep white matter ischaemia are isointense on the images (Schaefer, 2001). DWI can also help distinguish between acute haemorrhagic and nonhaemorrhagic stroke. Decreased ADC has been demonstrated in both acute haemorrhagic and in nonhaemorrhagic stroke. However, it was found that ADC remained decreased in haemorrhagic stroke even 100 days after the onset in contrast to nonhaemorrhagic stroke, where ADC increased after 31 days (Ebisu et al, 1997).

DWI may be able to identify early or more subtle abnormalities undetected by conventional MRI. Preliminary findings using DWI in a small sample of patients who suffered acute anoxic ischaemic encephalopathy following a range of conditions including cardiac arrest, carbon monoxide poisoning and respiratory failure which have been considered to mainly affect the grey matter, have been able to detect early white matter injury suggesting that white matter may be more vulnerable to ischaemia than previously considered (Chalela et al, 2001). DWI changes may also reflect the severity of neurological deficits with reports of a correlation between neurological assessment scales used in stroke and acute DWI lesion volume (Schwamm et al, 1998).

Epilepsy

DWI has also been applied in the study of epilepsy. Several case reports have detected acute DWI changes, namely reduction in diffusivity in seizure foci during ictal events (Wieshmann et al, 1997; Lansberg et al, 1999) and in status epilepticus (Ebisu et al, 1996; Flacke et al, 2000) reflecting cellular or cytotoxic oedema which appear to resolve when the patients recover. However, interictal DWI changes have also been reported in epilepsy and it has been suggested that DWI may help to delineate the epileptic focus. A recent study using the newer DTI sequences examined a group of patients with partial epilepsy of which 10 had acquired lesions and 30 had normal MRI findings (Rugg-Gunn et al, 2001). A voxel-based analysis revealed significant areas of increased mean diffusivity and reduced fractional anisotropy in patients with acquired lesions which corresponded to the abnormalities visualised on conventional MRI. In the MRI-negative group, a number of patients had areas of increased diffusivity or reduced anisotropy which were found to correspond to the localization of epileptiform EEG abnormality. These findings suggest that minor structural disorganization in epileptogenic foci can be detected with DWI and may contribute to presurgical assessments as conventional MRI is only able to identify structural lesions in about 80% of patients with refractory epilepsy. Another study which examined patients with partial epilepsy and malformations of cortical development using the newer DTI sequences, reported increased mean diffusivity and reduced fractional anisotropy in normal appearing tissue beyond the cortical malformations (Eriksson et al, 2001). These findings confirm that DWI is able to provide additional information to conventional MRI. The precise histopathological correlates of these findings are not known but the authors suggest that the increased diffusivity may reflect areas of reduced cell density and increased extracellular space due to failure of neurogenesis or

later cell loss whereas reduced anisotropy may be related to poor myelination or ectopic neurons in white matter or increased or abnormally located grey matter.

Demyelination/Multiple sclerosis

DWI has mostly been applied to demyelinating diseases such as multiple sclerosis as it is very sensitive to the direction of axons in white matter. In DWI, the high ADC in MS plaques is thought to mainly represent increased extracellular volume but the relative contributions from oedema or demyelination is not known. In addition, axonal loss may also contribute to increased ADC. Studies using the more recent DTI sequences have demonstrated that all MS lesions (acute and chronic) have significantly higher mean diffusivity and lower fractional anisotropy than NAWM (Werring et al, 1998). Acute MS lesions were found to have higher mean diffusivity and lower fractional anisotropy than chronic lesions. The range in the increase in mean diffusivity may reflect the different extent of oedema, demyelination and axonal loss (Werring et al, 1998) whereas reduction in fractional anisotropy is considered to reflect axonal degeneration (Ciccarelli et al, 2001).

It has been shown that DWI is not only able to identify MS lesions with severe tissue disruption but is also sensitive to more subtle structural damage in the NAWM not detectable with conventional MRI (Filippi et al, 2000a; Ciccarelli et al, 2001). A recent study measuring mean diffusivity in MS patients has demonstrated abnormalities in both normal appearing white and grey matter (Cercignani et al, 2001b). It is likely that the diffusion abnormalities in grey matter may reflect subtle MS lesions or wallerian degeneration of grey matter neurons secondary to the damage of fibres transversing MS white matter lesions (Filippi and Inglese M, 2001). Both diffusion measures, mean

diffusivity and fractional anisotropy, in NAWM have recently been found to correlate with physical disability in MS patients and thus may be of value in the evaluation of disease progression (Ciccarelli et al, 2001; Cercignani et al, 2001b). Furthermore, mean diffusivity in grey matter has been reported to correlate with neuropsychological test scores (Rovaris et al, 2000).

Tumour

DWI has been used in the evaluation of brain tumours. Earlier studies reported greater ADC in cerebral gliomas than in normal brain tissue. Furthermore, these studies found that ADC and diffusion anisotropy allowed the differentiation between various components of gliomas (enhancing, non enhancing, cystic, necrotic) as well as in distinguishing areas of nonenhancing tumour from areas of predominantly peritumoural oedema (Tien et al, 1994; Brunberg et al, 1995). A more recent study, however, did not find DWI to be superior to conventional MRI in the characterization of the different intracerebral tumours such as gliomas, metastases and meningiomas although there was some suggestion that it may be possible to differentiate them from cerebral lymphoma or abscess (Stadnik et al, 2001). In one study that used DWI to monitor response to treatment in patients with gliomas, mean ADC was found to increase with treatment and was correlated with clinical outcome (Chenevert et al, 2000).

DWI has also been used to study children with neurofibromatosis type 1. Increased ADC in hyperintense basal ganglia lesions as well as in normal appearing brain tissue have been reported in these patients (Eastwood et al, 2001).

Intracranial infection

DWI also allows the differentiation of cerebral abscesses from intracerebral necrotic tumours that is frequently impossible on conventional MRI. Abscesses appear hyperintense on diffusion-weighted images and have very low ADC values most likely due to the high cellularity and viscosity of pus (Schaefer et al, 2001). Therefore, the presence of a central area of hyperintensity on diffusion-weighted images and very low ADC values strongly suggest the presence of an abscess whereas the mass with central hypointensity and increased ADC values suggest cerebral glioma or metastasis.

A recent study has demonstrated that herpes encephalitis lesions are strongly hyperintense on DWI with low ADC due to the restricted diffusion consistent with cytotoxic oedema and can therefore be differentiated from gliomas which usually have increased ADC (Schaefer et al, 2001).

DWI has been used to detect subtle white matter abnormalities in a small sample patients with HIV-1 infection who did not have any clinical evidence of neurological disease or dementia (Pomara et al, 2001). Abnormal fractional anisotropy in white matter of the frontal lobes and internal capsules in these patients may represent the earliest manifestations of microstructural changes in white matter in HIV-1 disease.

DWI has also been applied in patients with Creutzfeldt-Jakob Disease (CJD) and has shown hyperintense lesions of varying ADC in the cortex and basal ganglia which are undetected on conventional MRI and may most likely be related to different degrees in spongiform changes, neuronal loss and gliosis (Bahn et al, 1997; Demaerel et al, 1999).

Trauma

DWI has been used in the study of acute brain trauma (Liu et al, 1999; Marmarou et al, 2000). One study reported reduced ADC in diffuse axonal injury lesions (Liu et al, 1999) up to 18 days after the traumatic injury indicating cellular or cytotoxic oedema.

Application in psychiatric disorders

The newer DTI sequences have recently been applied in psychiatry, specifically to schizophrenia, albeit in small samples. Most of these studies have explored the white matter tracts in schizophrenia. However, the methodologies used in these studies have been varied given that the technique is relatively new and the best ways of acquiring and analysing data have not yet been determined. Thus, the findings of these studies should be cautiously interpreted.

One of the first studies to apply DTI in the study of schizophrenia compared eight male patients with chronic schizophrenia and a median age of 48 years to a group of controls (Hedehus et al, 1998). A voxel-based analysis, Statistical Parametric Mapping (SPM96), was used to perform a groupwise comparison between the patients and controls on the fractional anisotropy maps in 18 oblique slices of 5 mm thickness which were spatially normalised to a template brain in Talairach space. The authors reported preliminary findings of reduced anisotropy in the corpus callosum which suggested either a reduction in the number of fibre bundles or disruption of the individual fibres within the white matter tracts. In another small study, Buchsbaum et al (1998) used DTI and PET to study a group of five patients (3 males and 2 females) with a mean age of 34 years and a group of 6 age and sex-matched controls. PET scanning was done on average about six months prior to DTI and both PET metabolic

data and DTI data were coregistered to anatomical MRI. A voxel-based analysis was used to examine relative anisotropy (RA), another DTI measure, in two axial slices intersecting the striatum that allowed visualization of the corpus callosum and major frontal white matter tracts. Significant reductions of RA in prefrontal white matter in schizophrenic patients were detected. In addition, this appeared to correspond to the fronto-striatal areas of abnormal metabolic activity on PET. The authors concluded that these results provide further evidence of a disruption in fronto-striatal connectivity in schizophrenia. However, these findings would need to be confirmed in a larger sample.

Lim et al (1999) used DTI to compare 10 US army male veterans with chronic schizophrenia and a mean age of 47.7 years to age-matched controls. In this study, a region of interest approach based on the analysis of 8 axial slices which included the corpus callosum and centrum semiovale was used to examine fractional anisotropy in the frontal, temporo-parietal and parieto-occipital regions. The results indicated significant widespread reductions in fractional anisotropy in white matter in the absence of volumetric abnormalities but not in the grey matter in the schizophrenic group.

Although the findings of the aforementioned three studies would suggest that white matter connectivity is compromised in schizophrenia, conflicting findings have been reported in more recent studies (Steel et al, 2001; Agartz et al, 2001). Steel et al (2001) used DTI and MRS to examine prefrontal white matter in a group of ten schizophrenic patients (5 males/5 females) with a mean age of 34 years who were compared to age and sex matched healthy controls. Using a region of interest approach, fractional

anisotropy (FA) and N-acetyl aspartate (NAA) were measured in the same prefrontal white matter region. There were no significant differences in FA between patients and controls but in contrast, there was a trend of reduced NAA in the prefrontal white matter. From these findings, the authors propose that abnormal connectivity in schizophrenia may not be attributable to structural abnormalities of white matter and that reduced NAA may reflect abnormal function of structurally intact neurons. In contrast to the earlier studies using small sample sizes and restricted number of brain slices for DTI analysis, the most recent study by Agartz et al (2001) used a larger sample size (20 schizophrenic patients and 24 healthy controls) and a voxel-based analysis to examine DTI changes in white and grey matter based on 22 axial slices which represented almost complete brain coverage. Fractional anisotropy was reduced in the splenium of the corpus callosum and occipital white matter in the absence of any volume deficits suggesting that the white matter tracts that transverse these areas and which may originate in the dorsoinferior parts of the temporal lobes and occipital lobes are abnormal in schizophrenia. This study also did not find any evidence of changes in fractional anisotropy in the prefrontal white matter though there were global increases in another diffusion measure, mean diffusivity, in both grey and white matter in the schizophrenic patients.

The findings of the DTI studies in schizophrenia to date have been inconsistent. Further studies in larger samples and using improved data acquisition and analysis are therefore needed to clarify the white matter abnormalities and may prove to be useful in the study of anatomical connectivity in schizophrenia.

CHAPTER 7

STUDY HYPOTHESES

Functional and structural imaging studies have recently suggested that schizophrenia may arise from abnormalities in the connections between different brain regions and not only from pathology within the regions themselves. This theory of abnormal brain connectivity has been applied in particular to fronto-temporal connections (Frith et al, 1995; McGuire and Frith, 1996a; Woodruff et al, 1997b). However, it has not been determined if abnormalities in connectivity are specifically related to pathological changes in white matter or the cortex.

Few neuropathological studies have examined the white matter in schizophrenia although there is some evidence that myelin abnormalities may be present. It has been suggested that abnormal myelination in the fronto-temporal fibres may be relevant in schizophrenia and may explain the onset of symptoms in early adulthood (Benes et al, 1994). More recently, a post mortem study in schizophrenic patients reported a down-regulation in the expression of myelination-related genes in the dorsolateral prefrontal cortex suggesting a dysfunction in myelin forming oligodendrocytes (Hakak et al, 2001). There is also indirect evidence for myelin abnormalities from MRS findings of a reduction in the concentration of choline containing compounds, many of which are myelin components, in the hippocampi of patients with chronic schizophrenia (Maier et al, 1995). Further support comes from reports of the high incidence of psychosis in patients with metachromatic leukodystrophy, an early onset demyelinating disease (Hyde et al, 1992).

Structural imaging studies in schizophrenia have detected reductions in cortical volumes, particularly in the frontal (Sullivan et al, 1998; Schlaepter et al, 1994; Goldstein et al, 1999; Wright et al , 1999), and temporal lobes (Pearlson et al, 1997; Lawrie and Abukmiel, 1998; Wright et al, 1999). These macroscopic findings have been confirmed by some post mortem studies (Pakkenberg 1987; Falkai et al, 1988; Bogerts et al, 1990) but the histopathological changes that underlie these abnormalities in the cortex have been more difficult to characterise. More recent histopathological studies have suggested that apart from neuronal abnormalities (Benes et al, 1991; Falkai et al, 1988; Akbarian et al, 1993), other subtle and widespread cytoarchitectural changes in the cortex may occur in schizophrenia. Studies using stereological techniques (Pakkenberg et al, 1993; Selemon et al, 1998) have suggested that subtle abnormalities in the neuropil, which includes neuronal processes such as dendrites and synapses, may also account for the cortical changes observed in schizophrenia (Selemon et al, 1998; Glantz and Lewis, 2000).

The three studies presented in this thesis are linked by the common aim of further investigating neuropathological changes in schizophrenia in vivo.

The following hypotheses were tested:

1. Abnormal connectivity in schizophrenia results from white matter abnormalities. Changes in the structural organisation of fibre tracts and abnormal myelination are likely to be the neuropathological basis of these white matter abnormalities.
2. White matter abnormalities in schizophrenia are likely to be detected in fronto-temporal regions and large fibre tracts such as the corpus callosum given that the

clinical and cognitive manifestations of the illness have been attributed to dysfunction of fronto-temporal circuits.

3. Cortical abnormalities compatible with the presence of neuropil abnormalities will also be detected and are likely to be the basis of altered cortico-cortical circuitry in schizophrenia. These abnormalities are more likely to occur in the fronto-temporal regions.

These hypotheses will be explored using MTI and DTI, which have the potential for providing more neuropathological information in vivo than conventional MRI. The relationship between MTR and DTI changes with clinical variables such as duration of illness or severity of schizophrenic symptoms will also be explored.

CHAPTER 8

RECRUITMENT OF SUBJECTS FOR THE STUDIES

Patients were recruited from the Bethlem and Maudsley Hospitals for the studies. Details about their past and current psychiatric and medical history, previous hospital admissions and treatments were obtained from the hospital medical records which were available for all patients and recorded in structured forms designed for the study. Controls were recruited from volunteers within the Institute of Neurology and National Hospital and from the local community where patients reside.

Inclusion criteria

Patients between the ages of 18 – 55 years, with a diagnosis of chronic schizophrenia that was confirmed following a clinical interview and upon reviewing their hospital records, were recruited. All patients fulfilled the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM IV) criteria for schizophrenia (24 with paranoid subtype; 1 with hebephrenic subtype) and were in a stable phase of their illness. Controls were selected to match the patient group as closely as possible with respect to age, gender and paternal social class (Goldthorpe, 1974).

Exclusion criteria

Any subject (patients and controls) with a history of neurological or systemic illness, head injury, a past history of alcohol intake of more than 30 units a week or drug abuse was excluded from the study.

The patient group

A total of twenty five patients (19 males, 6 females) with schizophrenia were recruited. Their age ranged between 25-46 years with a mean of 37.3 years. Their duration of illness ranged from 3-22 years with a mean of 14.3 years. All patients had a long history of previous treatment with antipsychotic medication and were on medication at the time of the study. Seven of the 25 patients were on atypical antipsychotics at the time of the study but had been treated with conventional antipsychotic medication in the past. The current daily dose of antipsychotic medication was calculated for each patient as equivalent to chlorpromazine according to the British National Formulary. All patients had previous psychiatric hospital admissions ranging from 1-10 episodes with a mean of 4 episodes.

The control group

A total of thirty healthy controls (22 males, 8 females) with a range of ages between 25-49 years and a mean of 35.1 years were recruited.

Informed consent was obtained from all subjects and the study was approved by the ethics committees of the Maudsley Hospital and Institute of Neurology.

CHAPTER 9

STUDY 1. MTR ABNORMALITIES IN WHITE MATTER IN SCHIZOPHRENIA

There is compelling evidence from functional and structural imaging studies that schizophrenia may be a disease of brain connectivity, in particular involving fronto-temporal connections. As discussed in Chapter 4, it is possible that myelin and axonal abnormalities may account for the disordered 'connectivity' but few studies have examined the white matter in schizophrenia. Most volumetric studies in schizophrenia have reported normal white matter volumes in contrast to widespread grey matter reductions (Harvey et al, 1993; Zipursky et al, 1992; Lim et al, 1996a) although punctate abnormalities in white matter have been detected by some investigators (Persaud et al, 1997). This suggests that in the absence of volumetric loss, the white matter abnormalities may involve subtle changes in myelin or the organisation and packing density of fibres.

Previous research has mainly focused on the frontal lobes with reports of more extensive areas of focal signal hyperintensities on MRI in schizophrenic patients compared to controls (Persaud et al, 1997) and post mortem abnormalities such as a selective maldistribution of interstitial neurons in prefrontal white matter (Akbarian et al, 1996a). There is also some recent evidence that white matter hyperintensities on MRI may be linked to affective symptoms and poor treatment response in patients with late onset depression (de Groot et al, 2000; Simpson et al, 1998) but it is unclear if the same applies to schizophrenia.

The aim of this study therefore was to determine whether subtle white matter abnormalities can be detected in schizophrenia. Different regions of white matter were examined in vivo in a group of schizophrenic patients and controls using MTI which is more sensitive to subtle structural changes than conventional MRI. This technique has not been previously applied to studies of patients with schizophrenia.

METHODS

Subjects

Twenty five patients (19 males, 6 females) who fulfilled the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM IV) criteria for schizophrenia and thirty healthy controls (22 males, 8 females) were selected for this study. Their demographical characteristics are described in Chapter 7.

Clinical assessments

i) The National Adult Reading Test (NART)

This test (Nelson and Willison, 1991) provided an estimate of premorbid IQ and was used to match patients and controls.

ii) Psychiatric symptoms

The subscales for positive and negative symptomatology of the Positive and Negative Syndrome Scale (PANSS)(Kay et al, 1987) were used to provide a measure of schizophrenic symptoms during the week prior to assessment. The PANNS is based on a standardised structured interview consisting of 30 items which takes about 30-40 minutes to administer. There are 3 subscales – the positive, negative and general psychopathology scales. Only the positive and negative subscales were used in this study. The positive scale measures 7 symptoms: delusions, conceptual disorganisation, hallucinatory behaviour, excitement, grandiosity, suspiciousness and hostility. The negative scale also measures 7 symptoms: blunted affect, emotional withdrawal, poor rapport, social withdrawal, difficulty in abstract thinking, lack of spontaneity or flow of conversation and stereotyped thinking. Each symptom is rated 1-7 depending on the severity (1=absent; 2=minimal; 3=mild; 4=moderate; 5=moderately severe; 6=severe

and 7=extreme). The potential total score for either the positive or negative scale range from 7-49.

iii) Neurological signs

The Annett questionnaire (Annett, 1970) was administered to assess handedness. This consisted of 14 questions used to determine hand preference.

Soft neurological signs were assessed in all subjects using the 'soft signs assessment' section (Part 2) of the Cambridge Neurological Inventory (Chen et al, 1995). This consisted of three groups of signs, namely 'primitive reflexes', repetitive sequential motor execution and integration of sensory information. The primitive reflexes tested were snout, grasp and palmomental reflexes. Sequential motor execution was assessed for each hand using finger-nose, finger-thumb tapping, finger-thumb opposition, mirror movements, diadochokinesis, fist-edge-palm and Osteretsky tests. Tests of sensory integration included rhythm tapping, go/no-go test, left-right orientation and finger agnosia, stereognosis and graphesthesia for each hand. Apart from the snout and palmomental reflexes (0=absent; 1=present), the scoring applied to the other tests ranged from 0-2 (0=no errors or normal; 1=one error indicating minor abnormality and 2=two or more errors indicating major abnormality). A total score for the three group of signs was calculated for each subject.

MRI

All subjects had a MRI scan which was performed on a GE Signa 1.5 Tesla scanner using a standard quadrature head coil and the total scanning time was approximately 60 minutes. The MT technique used in this study was based on a sequence developed

by Barker et al (1996) and has an advantage in that it minimises the effect of motion which may affect the calculation of MTR. The following sequences were used:

a. T2 weighted and proton density images were acquired initially using a dual echo sequence (TE [echo time] 15/90 ms, TR [repetition time] 3000ms, 28 contiguous 5mm axial slices, 256x256 pixel image matrix, 24x24cm^2 FOV [field of view]).
b. Imaging using a spin echo based magnetization transfer sequence (TE 30/80 ms, TR 1720 ms, 28 contiguous 5 mm axial slices, 256x128 pixel image matrix, 24x24cm^2 FOV) was acquired with and without a saturation pulse. The saturation pulse was a 16 ms, 23.2uT Hamming appodised 3 lobe sinc pulse, applied 1KHz from water resonance.

MT images were intrinsically coregistered with the proton density and T2 weighted images. MTR was calculated on a pixel by pixel basis as described in Chapter 5.

MRI and the clinical assessments were performed on the same day.

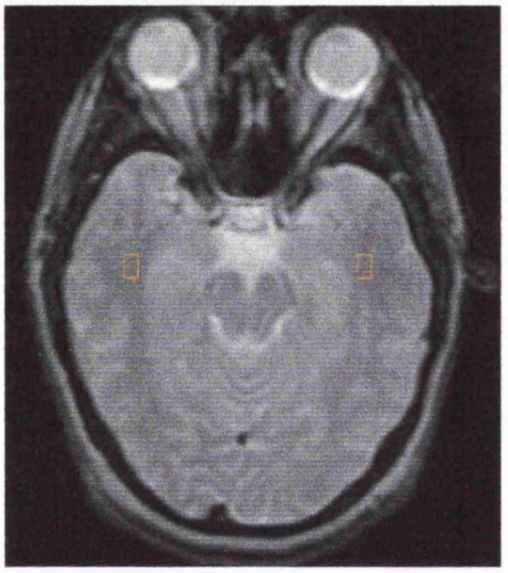
Data Analysis

A protocol was defined with guidance from a neuroradiologist for selecting regions of interest (ROIs) in the white matter based on the standard neuroanatomical divisions of the frontal, temporal, parietal and occipital lobes (Appendix 1). ROIs in the corpus callosum and white matter of the frontal, temporal, parietal and occipital regions of both hemispheres were sampled with reference to this protocol. Regions in the corpus callosum, frontal, temporal and parietal lobes were selected for this study as they have previously been reported to be abnormal in schizophrenia whilst the ROI in the

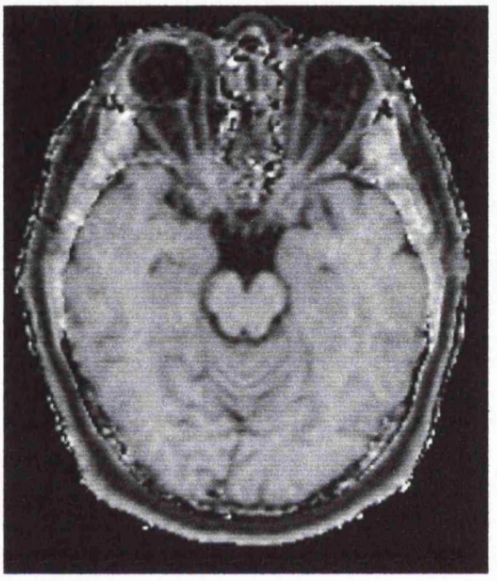
occipital lobe was chosen to provide an internal control region. Specifically, the temporal ROI was placed in the middle temporal gyrus and the frontal ROI was placed in the middle frontal gyrus to ensure that ROIs were strictly in white matter. The ROIs were standardised at 35.2 mm^2 and outlined on the T2 weighted images and not directly on the MT images to avoid any bias in placing them. Adjacent slices were checked to ensure that all ROIs were surrounded by white matter to minimise partial volume effects from grey matter and CSF. The interleaved MT images were co-registered with T2 weighted images which helps in selecting the regions of interest as T2 images provide good grey-white matter differentiation thereby reducing partial volume effects from grey matter or CSF. Selecting the regions on the T2 images also ensures that the rater is blind to the appearance of the calculated MTR images. The ROIs were then automatically transferred onto the MT images and mean MTR measurements were obtained in these regions. Figure 8 illustrates ROIs in the temporal white matter.

Figure 8. ROIs in temporal white matter

T2-weighted image



MTR image



RESULTS

The clinical and demographical information are summarised in Table 1.

Table 1. Clinical and demographical information for MTR study

	Schizophrenics (n=25)	**Controls** (n=30)	**t-test** *p* value
Age (years)	37.3 (6.7)	35.1(7.2)	ns
Gender (number of males/females)	19 / 6	22 / 8	
Premorbid IQ*	110.7 (12.2)	113.2 (7.8)	ns
Duration of illness (years)	14.3 (6.1)		
Number of psychiatric hospital admissions	4 (2)		
Dose of medication (mg /day) [chlorpromazine equivalent]	341.2		
PANNS			
Positive score	12.4 (4.6)		
Negative score	18.6 (6.2)		
Soft neurological signs	8.84	1.67	***p*<0.001**

Values are expressed as means (standard deviations)

* based on NART scores which were available for 19 patients and 19 controls

Demographical data

There were no significant group differences in age, gender and paternal social class between the schizophrenic patients and controls. There was also no significant group difference in mean premorbid IQ as estimated from the NART scores which were available for 19 patients and 19 controls (110.7 and 113.2 respectively). All subjects were right handed apart from one schizophrenic patient and two controls.

Illness duration and medications

The mean duration of psychiatric symptoms was 14.3 years (range 3-22 years) and all patients were on antipsychotic medication at the time of the study (mean dosage 341.2 mg per day equivalent to chlorpromazine, British National Formulary 2000). Seven patients were on atypical antipsychotic medications (3 = Clozapine; 3 = Risperidone and 1 = Olanzapine). The total medication dose since the onset of the illness was not calculated as it could not be accurately determined given that most patients had a long history of exposure to antipsychotic medication and variable compliance. All patients had previous psychiatric hospital admissions with a mean number of 4 episodes (range 2-10 episodes).

Psychiatric symptoms

Most patients scored higher on the negative than positive subscales of the PANSS. The mean total scores was 18.6 (range 9–33) for negative symptoms and 11.6 (range 7-24) for positive symptoms. These scores indicated that both negative than positive symptoms were only mild to moderate in severity for this group of patients.

Soft neurological signs

Schizophrenic patients had significantly greater total scores on the soft neurological signs scale compared to the controls (mean total scores of 8.84 and 1.67 respectively, z= -5.17, $p<0.001$). The patients scored higher than controls predominantly in the categories of motor coordination and sensory integration. The total score correlated significantly with the negative symptoms score (r=0.480; $p<0.01$) but not with age or duration of illness.

MTR analysis

The mean MTR values in the white matter were more variable and lower in almost all the regions in the schizophrenic patients compared to controls (Table 2).

A generalised linear mixed modelling (GLMM) approach (Hicks, 1982) was used to investigate group differences and examine the interactions between the variables avoiding multiple comparisons. This statistical model was considered appropriate as it allows data from a variety of continuous and discrete distribution to be linked to a linear structure that may contain both fixed and random factors. It can also account for interactions between observations as well as between random factors. The results of the GLMM analysis are shown in Table 3.

Table 2. Mean MTR values in white matter

Region	**Schizophrenics** (n=20)	**Controls** (n=20)	**t-test** *p* value
corpus callosum	39.00 (2.16)	39.46 (0.88)	ns
left frontal	39.63 (0.75)	39.62 (0.66)	ns
right frontal	39.33 (0.74)	39.24 (0.76)	ns
left parietal	38.49 (0.69)	38.78 (0.63)	ns
right parietal	38.60 (0.69)	38.73 (0.77)	ns
left temporal	39.17 (0.89)	39.95 (0.92)	***p*<0.01**
right temporal	38.59 (0.88)	39.67 (0.79)	***p*<0.001**
left occipital	37.38 (0.95)	37.78 (0.58)	ns
right occipital	37.58 (0.81)	37.44 (0.71)	ns

Values are expressed as mean (standard deviation) % units

Table 3. GLMM analysis for MTR

p-values for main effects and interactions

		***p*-values for Effects**						
		Group	Region	Side	Group x Region	Group x Side	Region x Side	Group x Region x Side
Full GLMM		**0.012**	**0.001**	**0.024**	**0.001**	0.664	**0.031**	0.784
Region by region GLMMs	*F*	0.768		**0.019**		0.856		
	P	0.938		0.988		0.521		
	O	0.437		0.543		0.342		
	T	**0.001**		**0.004**		0.704		
	CC	0.717						

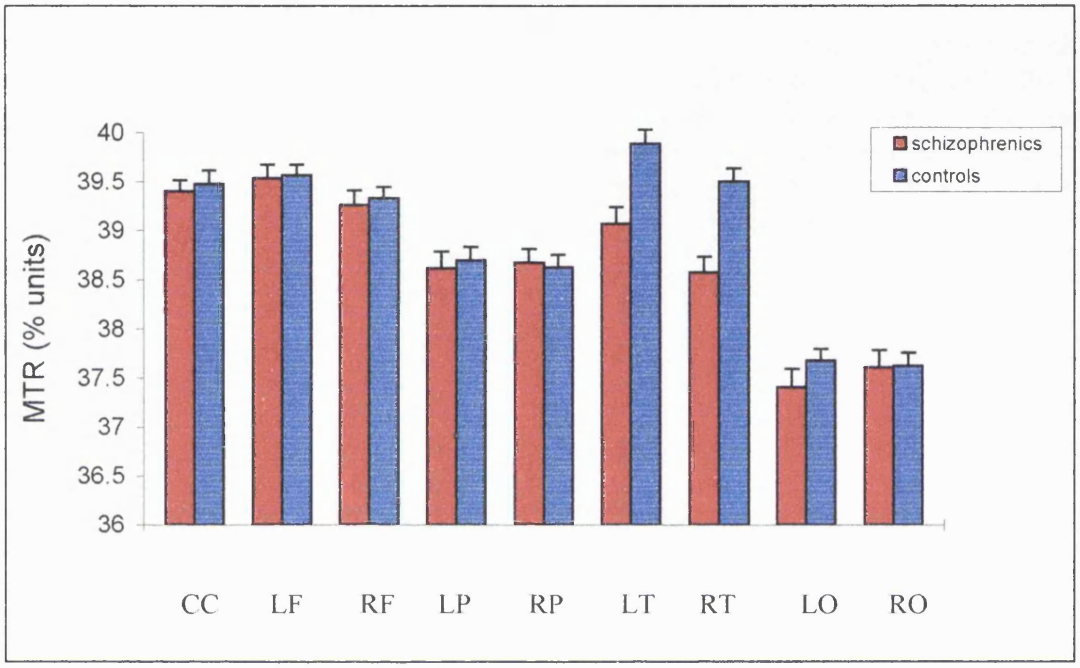
F frontal *P* parietal *O* occipital *T* temporal *CC* corpus callosum

Initially, a full GLMM was applied with fixed within subject factors *region* (frontal/parietal/occipital/temporal/corpus callosum) and *side* (left/right), a fixed between subjects factor *group* (control/schizophrenic) and a random factor *subject* (nested within *group*). The full model revealed that the MTR values, when all regions were considered together, were significantly different between schizophrenics and controls ($p<0.012$). The group x region interaction was highly significant ($p<0.001$) indicating that the group differences in MTR were dependent on the region. The region x side interaction was also significant ($p<0.031$) indicating that to a lesser extent, differences between right and left MTR values were dependent on the region. There was no significant group x side interaction indicating that the group differences in MTR were not dependent on the side.

Separate GLMMs were then applied to individual regions with one fixed within subject factor *side* (left/right), a fixed between subjects factor *group* (control/schizophrenic) and a random factor *subject* (nested within *group*). The individual region analyses revealed a highly significant group effect for MTR values in the temporal regions ($p<0.001$). This difference in mean MTR values represented about a 2% reduction in the schizophrenic group compared to the controls for the right temporal region (mean MTR of 38.58 and 39.51 respectively) and left temporal region (mean MTR 39.07 and 39.89 respectively) as shown in Figure 9.

Figure 9. MTR in white matter for the ROI analysis

mean (standard error of mean) values



CC corpus callosum; LF left frontal; RF right frontal; LP left parietal;

LT left temporal; RT right temporal; LO left occipital; RO right occipital

There was a significant effect of side for the temporal regions ($p<0.004$) but the group x side interaction was not significant indicating that right temporal MTR values were significantly different from the left in both the schizophrenic and control groups. No significant differences in frontal or other regional MTR values between schizophrenics and controls were detected. There was a significant effect of side for the frontal region ($p<0.019$) but the group x side interaction was not significant indicating that right frontal MTR values were significantly different from the left in both schizophrenics and controls.

Pearson's correlation coefficient was used to examine the association between regional MTR values within each group and to determine whether this association differed between the schizophrenic and control groups. There were some differences in the correlations between MTR values in the different white matter regions between the two groups, in particular, there were greater left frontal-right frontal and left frontal-left occipital correlations in the schizophrenic group compared to controls. A statistical test (Snedecor, 1989) of whether the correlations differed for the two groups indicated that only the left frontal-right frontal correlation was significantly different ($p<0.047$) suggesting that there may be subtle changes in frontal interhemispheric connections in the schizophrenic patients.

Exploring the clinical correlates of MTR

Using forward regression analysis, none of the clinical variables of age, duration of symptoms, PANSS scores and soft neurological signs predicted the temporal MTR values in the patient group. In addition, age did not predict MTR values in the control group.

It was important to explore the biological significance of the MTR findings and whether these were specific or may have been present in other regions which were not detected with the sample size in this study. Sample size calculations were therefore performed retrospectively to estimate the number of patients that would have been required to detect significant MTR changes in the different brain regions based on the total sample mean and standard deviations of the regional MTRs observed in this study, with a power of 80% and 5% level of significance. It was estimated that a sample size of 16 in each group would have been sufficient to detect statistically significant MTR changes in the temporal regions whereas at least 2000 subjects in each group would have been required to detect significant MTR changes in the frontal regions, if present. Sample size estimations for the other regions were similar to those for the frontal regions. This suggests that MTR abnormalities may be localised to the temporal regions and that if they are present in the other white matter regions sampled, they may be very subtle and of doubtful biological significance.

DISCUSSION

This study investigated white matter abnormalities in schizophrenia using a novel approach and the results indicate a significant reduction of MTR in temporal white matter in schizophrenic patients compared to controls which is likely to reflect myelin and/or axonal disruption.

MTR findings

Large reductions in MTR are observed in lesions with major myelin or axonal loss such as in multiple sclerosis but smaller reductions could be due to more subtle abnormalities in either myelin or axons. Some studies of the temporal lobes in

schizophrenic patients have reported changes in neuronal density in the hippocampus (Zaidel et al, 1997; Benes et al, 1998) and this could lead to axonal disruption. There is also evidence of a reduction in N-acetyl aspartate (NAA), considered to be a marker of neuronal or axonal integrity, in the temporal lobes (Yurgelon-Todd et al, 1996; Renshaw et al, 1995) and hippocampus (Maier et al, 1996; Bertolino et al, 1996). More recently, Lim et al (1998) reported a reduction in NAA signal intensity in brain white matter without any volumetric change in a group of schizophrenic patients and suggested that this may reflect a disruption of axonal connections. In addition, the indirect evidence that myelin abnormalities may be present in the hippocampus (Maier et al, 1995; Benes et al, 1994) and the high incidence of psychosis in metachromatic leukodystrophy patients (Hyde et al, 1992) gives further support to this possibility. It is therefore possible that subtle changes in axonal and myelin structure and/or chemistry could have contributed to the MTR reduction in the schizophrenic patients in this study.

The finding of MTR abnormalities circumscribed to temporal lobe white matter and the absence of any significant correlation between frontal and temporal MTR values lends little support to the recent suggestion of a disruption of anatomical connections between different brain regions, particularly of the fronto-temporal connections in schizophrenia (Woodruff et al, 1997b). However, it remains possible that MTR abnormalities may be present in other regions not sampled in this study. It is difficult to draw firm conclusions from the observation of significantly different correlations between frontal MTR values in schizophrenic patients compared to controls. Subtle changes in interhemispheric connections may be responsible, but it is possible that the

greater variability of MTR values in the schizophrenic patients may have accounted for the difference.

Clinical covariates

Age and duration of illness failed to predict MTR changes in the schizophrenic patients suggesting that these changes are unlikely to be progressive although this would need to be confirmed in longitudinal studies. Neither positive nor negative schizophrenic symptoms predicted the MTR changes in patients suggesting that they may be common to the different clinical subtypes. The same applies to the presence of soft neurological signs which were significantly more common in schizophrenic patients and associated with the presence of negative symptoms.

Methodological considerations

The ROI approach is an appropriate method for examining the associations between different regions, particularly the frontal and temporal areas that have been suggested to be abnormal in schizophrenia. However, it is possible that the findings in this study may have been limited by the use of this methodology which may have missed more subtle MTR changes elsewhere.

CHAPTER 10

STUDY 2: DTI CHANGES IN WHITE MATTER IN SCHIZOPHRENIA

White matter abnormalities have been reported in schizophrenia particularly in the corpus callosum (Woodruff et al, 1995; Highley et al, 1999) and frontotemporal (Akbarian et al, 1996a; Persaud et al, 1997) regions. A meta-analysis (Woodruff et al, 1995) of MRI studies of the corpus callosum has established that the majority have reported a reduction in the area of the corpus callosum in schizophrenic patients compared to controls. However, less is known about the underlying histopathological changes or the functional significance of these structural abnormalities. Earlier post mortem studies (Casanova et al, 1989; Nasrallah et al, 1983) found no abnormality in axonal counts but a more recent study has reported disruption of axonal density in most areas of the corpus callosum in female schizophrenic patients (Highley et al, 1999).

Diffusion tensor imaging (DTI) is capable of detecting more subtle changes in the nature and organization of white matter tracts in vivo that are not detected with conventional MRI. To date, only a few studies have used DTI to investigate the integrity of white matter tracts in vivo in schizophrenia and only in very small samples.

The aim of this study was to explore structural abnormalities in white matter using DTI in a group of schizophrenic patients. Two techniques were used to analyse the DTI data:

1. A ROI methodology was chosen to investigate the corpus callosum because it is a large fibre tract that can be easily identified and is large enough for the ROI to be reliably placed.
2. A voxel-based analysis was used to investigate other white matter regions. This was considered more appropriate than a ROI approach because of the intersubject variability in the directionality and division of fibre tracts.

METHODS

Subjects

Subjects for this study were recruited from the sample of patients and controls described in Chapter 7. This consisted of twenty patients (15 male, 5 female) with schizophrenia whose ages ranged between 26-46 years and a mean duration of psychiatric symptoms of 13.75 years. All patients had a history of exposure to neuroleptic medication and were on antipsychotic medication at the time of the study. Twenty five controls (16 male, 9 female) with an age range of 22-49 years were selected to match the patient group with respect to gender and age.

Clinical assessments

Psychiatric symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al, 1987). A full description of the PANSS is given in Study 1.

The Annett questionnaire (Annett, 1970) was used to assess hand preference.

MRI

DTI was performed on a Signa 1.5 Tesla scanner (GE, Milwaukee, USA) equipped with shielded magnetic field gradients of up to 22 mT/m. A standard quadrature head coil was used for RF transmission and reception of the NMR signal. Head motion was minimised with standard foam pad immobilisation as provided by the manufacturer. Diffusion-weighted echo planar images (DW-EPI) were acquired in the axial plane (TE=78ms, 96 x 96 matrix, field of view = 24cm x 24cm, slice thickness = 5mm) at 12 slice locations centred on the lateral ventricles. The diffusion-sensitising gradients were applied along 7 non-collinear directions at 4 gradient strengths corresponding to

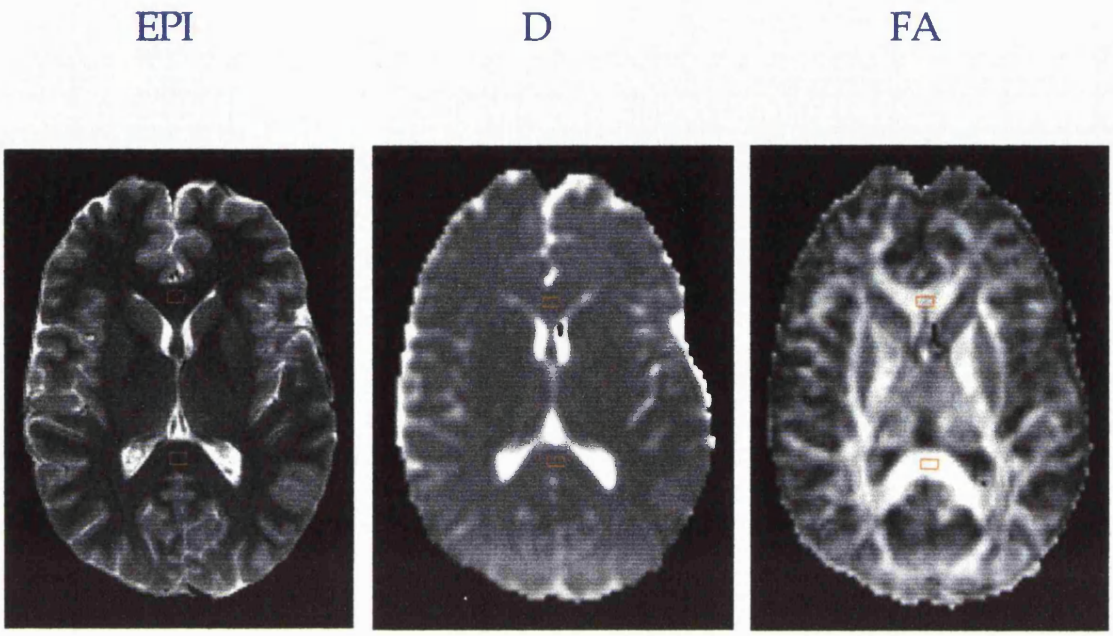
b values from 0 to 700 s/mm^2. Images were acquired in two sets of 6 contiguous interleaved slices due to image storage limitations. Five acquisitions of each set were performed and co-added following magnitude reconstruction to improve signal to noise ratio. A total of 840 images were acquired in each of the two acquisitions per subject. The average signal-to-noise ratio on the images with maximum diffusion weighting was 25:1. Cardiac gating was utilised via a peripheral pulse oximeter; images acquisition was triggered from every second R wave to reduce signal modulation due to brain pulsation. Maps of the diffusion tensor elements, mean diffusivity (D) and fractional anisotropy (FA) were calculated on a pixel by pixel basis as described by Basser et al (1994).

Image analysis

1. ROI analysis

Images were displayed on a Sun workstation and the regions of interest (ROIs), standardised at 28.1 mm^2, were outlined on the non-diffusion weighted (b=0) echoplanar images and not directly on the DTI to avoid any bias in placing them. This ROI analysis was only applied to the corpus callosum and the ROIs were placed in the genu (anterior) and splenium (posterior) of the corpus callosum on the slice in which they were visualised to be of maximal thickness. Adjacent slices were checked to ensure that partial volume effects from CSF were minimised. As DTI images were co-registered with the echoplanar images, the ROIs were automatically transferred to the corresponding diffusion maps to obtain D and FA measurements (Figure 10).

Figure 10. ROIs in the corpus callosum for DTI



EPI = echoplanar image; D = mean diffusivity map; FA = fractional anisotropy map

2. Voxel-based analysis

Images were displayed on a Sun SPARC workstation (Sun Microsystems, Mountain View, CA) and 6 contiguous slices above the anterior commissure encompassing corpus callosum and centrum semiovale were used for the analysis in Statistical Parametric Mapping [SPM96] (Wellcome Department of Cognitive Neurology, London, UK) (Friston et al, 1995b). SPM is one of the most widely used voxel-based analysis software originally developed to analyse PET data but has also been shown to be sensitive in analysing structural MRI data (Woermann et al, 1999) and more recently DTI data (Rugg-Gunn, 2001; Eriksson et al, 2001). SPM refers to the construction of spatially extended statistical processes to test hypotheses about regional specific effects. SPMs are image processes with voxel values that are, under the null hypothesis, distributed according to a known probability density function (usually Gaussian). These statistical parametric maps are 3D projections of statistical functions that are used to characterise significant regional brain differences in imaging parameters.

The b0 images were transformed using Generic Brain Activation Mapping (GBAM) (Brammer et al, 1997) into a common space defined by a whole-brain scan of a normal control. The scan of the normal control was transformed into Talairach space using SPM96. The fractional anisotropy and mean diffusivity files were then transformed into Talairach space, and a threshold was applied to mask out the signal from the scalp. GBAM registration succeeded in 14 patients and 19 controls from DTI data available in the group of 20 patients and 25 controls. The failure to register the DTI data for all subjects may have been due to the limited number of slices acquired for DTI and those that failed were excluded from the analysis. Fractional anisotropy and mean diffusivity

maps were smoothed using a Gaussian filter to improve signal to noise ratio and to allow for intersubject anatomical variability.

Statistical Analysis

1. ROI analysis

Non-parametric tests (Mann-Whitney, Spearman's correlation coefficient) were used for group comparisons and the correlation analysis. Regression analysis was applied to determine if clinical variables predicted the DTI changes in the corpus callosum.

2. Voxel-based analysis

SPM96 was used to examine group comparisons of FA and D between schizophrenic patients and controls for other areas of white matter. A statistical threshold ($z=3.09$, $p<0.001$) was used to identify areas of significant differences between the groups and a correction for multiple comparisons was made (corrected p value < 0.05).

RESULTS

1. ROI analysis

The clinical and demographical data are summarised in Table 4. There were no significant differences in age, gender and paternal social class between the schizophrenic patients and controls. Most patients scored higher on the negative than positive subscales of the PANSS and the scores indicated that they were both only mild to moderate in severity. All subjects were assessed to be right handed apart from one patient and one control.

Table 4. Clinical and demographical data for the DTI study in the corpus callosum

	Schizophrenics (n=20)	**Controls** (n=25)
Age (years)	37.6 (6.5)	33.8 (7.6)
Gender (number of males/ females)	15/5	16/9
Duration of illness	13.8 years (5.6)	-
PANNS scores Positive Negative	 13.05 (4.6) 19.60 (6.2)	 - -
Dose of medication (chlorpromazine equivalent in mg/day)	367.5 mg	-

Values are expressed as means (standard deviations).

D was significantly increased and FA significantly reduced in the splenium of the corpus callosum in the schizophrenic group compared to controls (p <0.01 and p<0.02 respectively). However, D and FA in the genu did not significantly differ between the two groups. These results are shown in Table 5.

There were no significant gender differences in D and FA for either the schizophrenic or control group. Using forward regression analysis, none of the clinical variables, namely, age, duration of illness, dose of antipsychotic medication, positive or negative schizophrenic symptoms predicted the DTI changes in the corpus callosum in the schizophrenic group.

Table 5. Mean diffusivity and fractional anisotropy in the corpus callosum

	Schizophrenics (n=20)	**Controls** (n=25)	**Mann-Whitney**
D (splenium)	0.943 (0.138)	0.840 (0.073)	z= -2.51, ***p*<0.01**
D (genu)	0.808 (0.067)	0.804 (0.053)	ns
FA (splenium)	0.776 (0.047)	0.820 (0.067)	z= -2.36, ***p*<0.02**
FA (genu)	0.820 (0.057)	0.823 (0.047)	ns

Values are expressed as means (standard deviations)

D mean diffusivity ($x10^3$ mm^2/s); FA fractional anisotropy (dimensionless units)

2. Voxel-based analysis

A subgroup of subjects was used for the voxel-based analysis. This consisted of fourteen (11 male, 3 female) patients with chronic schizophrenia with an age range between 27-46 years. Nineteen healthy controls (12 male, 7 female) with an age range between 25-49 years were selected to match this patient group with respect to gender and age. The demographical and clinical data are summarised in Table 6.

Table 6. Clinical and demographical data for the DTI study in regional white matter

	Schizophrenics (n=14)	**Controls** (n=19)
Age (years)	38.6 (6.7)	34.6 (8.0)
Gender (number of males/ females)	14/3	12/7
Duration of illness	13.7 years (5.9)	-
PANNS Positive Negative	 13.4 (5.6) 20.1 (6.7)	
Dose of medication (chlorpromazine equivalent in mg/day)	326.9 mg	

Values expressed as means (standard deviations)

There were no significant differences in age, gender and paternal social class between the schizophrenic patients and controls. The PANNS scores indicated that positive and negative symptoms were only mild to moderate in severity in this group of patients. All subjects were assessed to be right handed apart from one patient and one control.

Using a range of smoothing and grey matter thresholds, no areas of significant differences in FA or D in regional white matter were detected between patients and controls at the cluster (group of voxels) or voxel level. There were also no significant differences in FA or D in grey matter between patients and controls. A summary of the range of smoothing and grey matter thresholds applied for the group comparison of FA is shown in Table 7.

Table 7. Range of smoothing and grey matter (GM) thresholds for the group comparison of FA

Smoothing	GM threshold	corrected *p*-value		uncorrected *p*-value	x, y, z (mm)
		cluster - level	voxel- level		
6	0.4	0.361	0.744	0.009	11 17 0
		0.672	0.827	0.084	-9 -92 15
		0.914	0.881	0.084	-2 -28 0
		0.998	0.998	0.118	41 -86 -5
		0.980	0.999	0.185	22 -66 20
	0.8	0.551	0.765	0.025	11 17 0
		0.873	0.637	0.100	-9 -92 15
		0.969	0.960	0.153	-2 -28 0
		0.976	0.969	0.179	11 -99 10
		0.975	0.985	0.139	19 -62 15
8	0.4	0.791	0.767	0.185	-9 -92 15
		0.722	0.773	0.110	11 19 0
		0.830	0.810	0.273	19 -64 15
		0.865	0.850	0.281	9 -99 10
		0.924	0.916	0.289	-2 -32 -5
		0.938	0.997	0.252	-11 -101 0
	0.8	0.449	0.509	0.106	11 19 0
		0.573	0.550	0.268	19 -64 15
		0.710	0.696	0.284	-2 -32 -5
10	0.2	0.751	0.735	0.226	-9 -92 15
		0.793	0.822	0.242	11 19 0
		0.832	0.941	0.275	-9 -98 -5
	0.4	0.721	0.705	0.221	-9 -92 15
		0.759	0.796	0.237	11 19 0
		0.800	0.926	0.270	-9 -98 -5
	0.6	0.645	0.627		21 -64 20
		0.672	0.656		9 -92 15
		0.709	0.695		9 -98 10
		0.701	0.751		11 19 0
		0.829	0.823		-2 -32 -5
		0.746	0.897		-9 -98 -5
	0.8	0.551	0.606	0.227	11 19 0
12	0.4	0.273	0.585	0.084	-9 -94 15
	0.8	0.778	0.749		45 -88 -5
		0.820	0.796		-9 -92 15
16	0.4	0.135	0.275	0.092	-17 -103 5
	0.8	0.169	0.208	0.170	-17 -103 5

DISCUSSION

In this study, DTI provided quantitative measures of directionally averaged diffusion (mean diffusivity) and diffusion anisotropy (fractional anisotropy). These two complementary measures, D and FA, are considered to be sensitive indices of axonal integrity. Increased mean diffusivity has been reported in multiple sclerosis lesions and normal appearing white matter which may reflect oedema, demyelination and axonal loss (Werring et al, 1998). Reduced fractional anisotropy provides further evidence of structural damage in white matter and may reflect a disruption in the organisation of tracts (Werring et al, 1998; Wieshmann et al, 1998).

Corpus callosum findings

DTI was able to demonstrate that neuropathological abnormalities in the corpus callosum are present in schizophrenia. D and FA changes were detected in the splenium but not the genu of the corpus callosum suggesting that these changes may be focal.

A few studies to date have used DTI to investigate the white matter in schizophrenia but have only been performed in small samples. This study confirms previous findings of reduced FA in the corpus callosum (Hedehus et al, 1998; Agartz et al, 2001) and in particular, the localisation to the splenium (Agartz et al, 2001).

For this study, age-matched controls were selected to compare with the patients as it has been demonstrated that there are age related changes in FA (Virta et al, 1999). The reduction in FA and increase in D in the splenium of the corpus callosum suggest a disruption of axonal integrity likely to be related to the density or organisation of

fibres. It is possible that DTI changes may also reflect myelin abnormalities. However, a recent study (Miranda et al, 1998) demonstrated similar FA values in partially myelinated or unmyelinated white matter structures in the infant brain compared to the fully myelinated adult brain indicating that the contribution of myelin abnormalities to changes in anisotropy may be less significant than that from axonal abnormalities.

The corpus callosum was examined using the ROI methodology in this study as it is a large fibre tract that can be easily identified and is large enough for the ROI to be reliably placed with minimal intersubject variability in the directionality and division of fibre tracts. In the two areas of the corpus callosum sampled in this study, abnormalities were only present in the splenium. It has been demonstrated that the interhemispheric fibres from the inferotemporal and occipital lobes transverse the splenium whereas those from the frontal lobes transverse the genu (Pandya et al, 1986). It is therefore possible that the DTI changes in the splenium may have resulted from a focal disruption in the cortical neurons or axons in these areas. Although it is possible that DTI abnormalities may have been present in wider regions of the corpus callosum, the absence of changes in the genu suggest that these abnormalities may be focal within the corpus callosum. Gender differences in axonal density in some areas of the corpus callosum have been reported (Highley et al, 1999), however, such differences could not be detected in this sample which may be due to the small number of females.

Age and duration of illness failed to predict the DTI changes in the corpus callosum in schizophrenic patients suggesting that these abnormalities are unlikely to be progressive although this would need to be confirmed by longitudinal studies.

Likewise, positive and negative symptoms did not predict the DTI changes suggesting that they may be common to the different clinical subtypes. The findings are unlikely to be medication related given that DTI changes were only restricted to one of the two areas of the corpus callosum sampled and in addition, the dose of antipsychotic medication did not predict the DTI changes in these patients. An attempt was made to minimise the possibility of alcohol and drug abuse as confounding factors by using this as one of the exclusion criteria for the study.

Thus, the DTI findings in the corpus callosum suggest that there may be a focal disruption of commissural connectivity in schizophrenia.

Other white matter and grey matter findings

A voxel-based analysis was performed to determine whether DTI changes could be detected in other white matter regions. However, the registration of DTI data was only possible in a subgroup of patients (14 out of 20 patients) and controls (19 out of 25). This analysis was unable to demonstrate any differences on the DTI measures in regional white matter between the schizophrenic patients and controls. The results suggest that the structural integrity in regional white matter was not disrupted in this group of patients.

The findings in this study do not support previous reports of widespread (Lim et al, 1999) and more specifically prefrontal (Buchsbaum et al, 1998) white matter reductions in anisotropy in schizophrenic patients. However, these findings may not be comparable to those of the other studies as different methods of data acquisition and analysis were used. In this study, a voxel-based analysis was used to examine FA and

D in 6 slices that included the corpus callosum and centrum semiovale. The sample also included both male and female patients. In contrast to this study, Lim et al (1999) used a region of interest approach to examine FA in 8 axial slices in a smaller sample of ten patients. In addition, all their patients were male and significantly older (47.7 years) than the patients in this study. The other study by Buchsbaum et al (1998) which used a voxel-based analysis to examine relative anisotropy (RA), another DTI measure, in 2 axial slices, was also performed in a very small sample of schizophrenic patients (3 males and 2 females). Furthermore, there have been conflicting reports with two more recent studies failing to detect any significant differences in fractional anisotropy in prefrontal white matter between schizophrenic patients compared to controls using both a region of interest approach (Steel et al, 2001) and voxel-based analysis (Agartz et al, 2001).

The findings in this study are also in contrast to those by Agartz et al (2001) who reported global increases in mean diffusivity in both white and grey matter in their group of schizophrenic patients. This may be attributable to methodological differences with the larger sample size and greater brain coverage used in their study.

An unexpected finding was that the voxel-based analysis failed to confirm the ROI findings of subtle focal DTI abnormalities in the corpus callosum. However, this may have been due to the smaller sample size as the voxel-based analysis could only be applied to a subgroup of patients. In addition, the normalization and smoothing procedures may have reduced the detection of subtle changes. This suggests that a ROI methodology may be more sensitive in detecting DTI changes in large fibre tracts that can be easily identified and large enough for the ROI to be reliably placed. However, it

may be less appropriate for regional white matter due to the intersubject variability in the directionality and division of fibre tracts.

An important consideration is the application of SPM statistics to DTI analysis. It has recently been suggested that the use of cluster size to assess significance in SPM is only appropriate if the data are normally distributed (Ashburner and Friston, 2000) and therefore using the voxel level of significance as done in this study may be more appropriate. The findings in this study are by no means conclusive, given that this study may have been limited by the small sample size and restricted number of brain slices examined in the analysis.

CHAPTER 11

STUDY 3: MTR ABNORMALITIES IN THE CORTEX IN SCHIZOPHRENIA

Structural imaging studies in schizophrenia have detected reductions in cortical volumes, particularly in the frontal (Sullivan et al, 1998; Schlaepter et al, 1994; Goldstein et al, 1999; Wright et al , 1999), and temporal lobes (Pearlson et al, 1997; Lawrie and Abukmiel, 1998; Wright et al, 1999). These macroscopic findings have been confirmed by some post mortem studies (Pakkenberg 1987; Falkai et al, 1988; Bogerts et al, 1990). However, it has been more difficult to characterise the histopathological changes that underlie these abnormalities. It is well recognised that a number of methodological problems such as small sample size, effects of ageing and differences in histological techniques have limited such studies. More recent histopathological studies suggest that apart from neuronal abnormalities, other subtle and widespread cytoarchitectural changes in the cortex may occur in schizophrenia.

The purpose of this study therefore was to determine whether subtle structural abnormalities in the cortex in schizophrenia can be detected using MTR given that the technique has been found to be sensitive in detecting abnormalities in normal appearing brain tissue that are undetected on conventional MRI. A voxel-based analysis was adopted to examine MTR changes in the brain globally without any *a priori* assumptions of regional abnormalities in a group of schizophrenic patients compared to controls and to explore the relationship between cortical MTR changes and clinical variables.

METHODS

Subjects

There were twenty-five patients with chronic schizophrenia and thirty healthy controls involved in this study. A full description is given in Study 1.

Clinical assessments

Described in Study 1.

MRI

The scanning protocol has been described in Study 1.

Data Analysis

Data were analysed on a Sun SPARC workstation (Sun Microsystems, Mountain View, CA) using SPM96 (Wellcome Department of Cognitive Neurology, London, UK) (Friston et al, 1995b). The following steps were used to process and analyse the data:

1. Spatial normalization

The T2 images were registered into a standard (Talairach) space such that they all conform to a standard brain which facilitates intersubject averaging. A 3-stage process using a modified version (Symms et al, 1996) of the Automated Image Registration software (Woods et al, 1992) was applied as follows:

a. The T2-weighted images, including skull, were registered to the Montreal Neurological Institute (MNI) T2-weighted average brain that is provided with SPM96.

b. An automated program was then used to mask the registered images with a version of the MNI atlas where most of the skull had been removed from the T2-weighted image.

c. The skull-stripped T2-weighted image was then registered to the skull-stripped T2-weighted MNI atlas.

Following this process, the two registration parameter files were combined and applied to the MTR images, transforming them into Talairach space. The skull was removed by masking with the skull-stripped MNI atlas.

2. Smoothing

Spatial smoothing was then applied to the images using a 10 mm FWHM Gaussian filter (SPM 96) to improve signal to noise ratio and so that the data conformed more closely to a Gaussian field model which allows for intersubject anatomical variability.

3. Statistical analysis

Following the above processing steps, a group comparison of MTR changes between schizophrenic patients and controls was performed. The estimates were compared using two contrasts and grey matter threshold was set at 40%. For each voxel, this analysis detected the probability of MTR changes (i.e. a reduction or increase in MTR) in schizophrenic patients relative to controls. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the t statistic (SPM {t}). The SPM maps were then transformed to the unit normal distribution to give a Gaussian Field or parametric maps of the Z-statistic (SPM {Z}) from which p values were derived. A statistical threshold of $z=3.09$, $p<0.001$ was used to identify areas of significant

differences in MTR between the groups at the cluster (group of voxels) and individual voxel level. The use of cluster size to assess significance in SPM is considered appropriate if the data are normally distributed (Ashburner and Friston, 2000) otherwise the voxel level of significance should be used. To correct for multiple comparisons, the resulting foci were characterised in terms of spatial extent k (Friston et al 1995a). This correction describes the probability that a region of the observed number of voxels could have occurred by chance over the entire volume analysed (i.e. a corrected p value) (Friston et al, 1995a). The corrected p value selected was $p < 0.05$.

As 3-dimensional volume data sets were not acquired at the time of the study, the proton density weighted images from the MT sequence which are sensitive to volumetric changes were analysed to determine if there was significant cortical volume reduction that may have contributed to the MTR changes. A group comparison of the proton density images was performed using the same process as for MTR, to identify areas of significant reduction in signal intensity that would suggest reduction of cortical volume.

4. Segmentation

To determine whether the areas of MTR changes were located in white or grey matter, the *a priori* grey and white matter probability maps provided with SPM99 were used to compare with the normalised MR images. A threshold of 50% on the white matter probability map was chosen to identify areas more likely to be in white matter than grey matter.

RESULTS

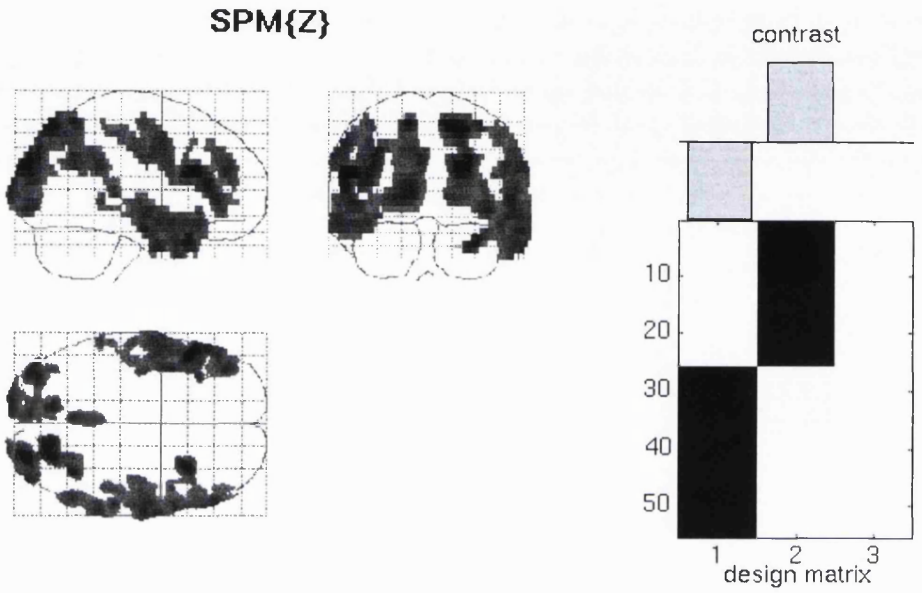
The demographic and clinical data for patients and controls have been reported in Study 1 and shown in Table 1.

Group differences in MTR

Group analysis in SPM was initially done to compare half the controls to the other half in order to determine if any differences could be detected with the level of smoothing, grey matter threshold and level of significance used. As no significant MTR changes were detected between the two groups of controls, these same levels of smoothing, grey matter threshold and level of significance were thereby considered appropriate for examining differences between patients and controls. Group analysis comparing the schizophrenic patients to controls revealed bilateral areas of significant MTR reduction at the cluster level ($p<0.05$) in schizophrenic patients in inferior and middle frontal, inferior and middle temporal and superior occipital gyri (Figure 11).

Of these areas, the left inferior frontal, right superior occipital and right inferior temporal were also significant at the voxel level. Using the segmented white matter map, the areas of MTR reduction were identified to be predominantly in the cortex, particularly in frontal and temporal regions. The MTR reductions extended into the white matter only in the temporal lobes, specifically in the middle temporal gyri (Figure 12).

Figure 11. MTR differences between schizophrenics and controls



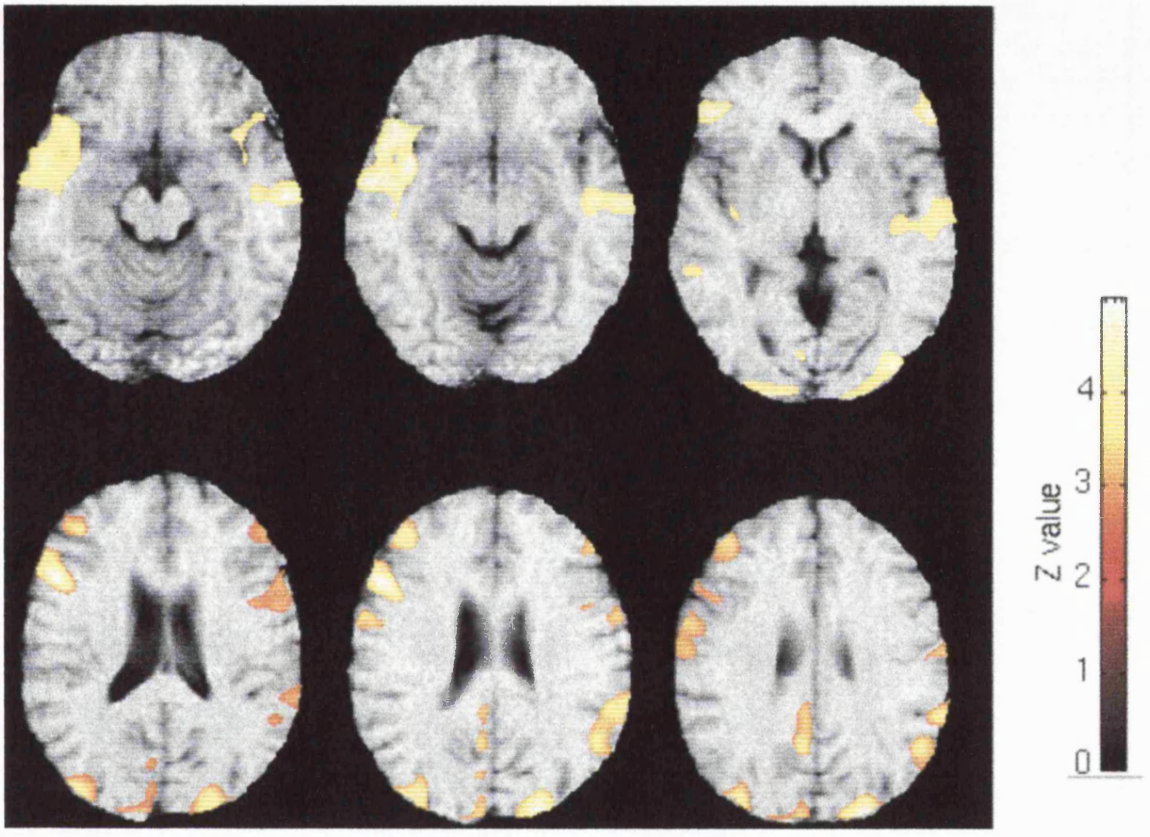
P values & statistics:

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z		x,y,z {mm}
0.000 (10)	**0.002 (1780, 5.46)**	**0.001 (5.46)**	**0.000**	**0.000**	**-52 21 20**
		0.046 (4.48)		0.000	-57 31 15
		0.113 (4.25)		0.000	-52 38 0
	0.000 (3734, 5.29)	**0.001 (5.29)**	**0.000**	**0.000**	**26 -93 30**
		0.004 (5.08)		0.000	14 -76 60
		0.006 (4.96)		0.000	23 -77 55
	0.000 (5486, 5.10)	**0.003 (5.10)**	**0.000**	**0.000**	**31 17 -30**
		0.077 (4.35)		0.000	65 -11 -20
		0.108 (4.26)		0.000	66 -2 -35
	0.000 (3534, 4.68)	**0.021 (4.68)**	**0.000**	**0.000**	**-13 -75 60**
		0.061 (4.42)		0.000	-38 -89 25
		0.065 (4.40)		0.000	-36 -85 35
	0.007 (1462, 4.35)	**0.078 (4.35)**	**0.001**	**0.000**	**60 38 0**
		0.239 (4.02)		0.000	61 32 10
		0.455 (3.79)		0.000	62 18 15
	0.025 (981, 4.26)	**0.110 (4.26)**	**0.004**	**0.000**	**-40 44 25**
		0.638 (3.62)		0.000	-44 39 35
		0.939 (3.27)		0.001	-41 33 45
	0.000 (3326, 4.17)	**0.147 (4.17)**	**0.000**	**0.000**	**-61 -3 -15**
		0.153 (4.16)		0.000	-41 7 -15
		0.195 (4.09)		0.000	-49 25 -15

Height threshold {u} = 3.09, p = 0.001
Extent threshold {k} = 6.926663e+02 voxels, p = 0.012
Expected voxels per cluster, E{n} = 97.7
Expected number of clusters, E{m} = 0.1

Volume {S} = 434010 voxels or 471.2 Resels
Degrees of freedom due to error = 53.0
Smoothness {FWHM mm} = 14.9 15.3 17.7
{voxels} = 6.3 6.5 7.5

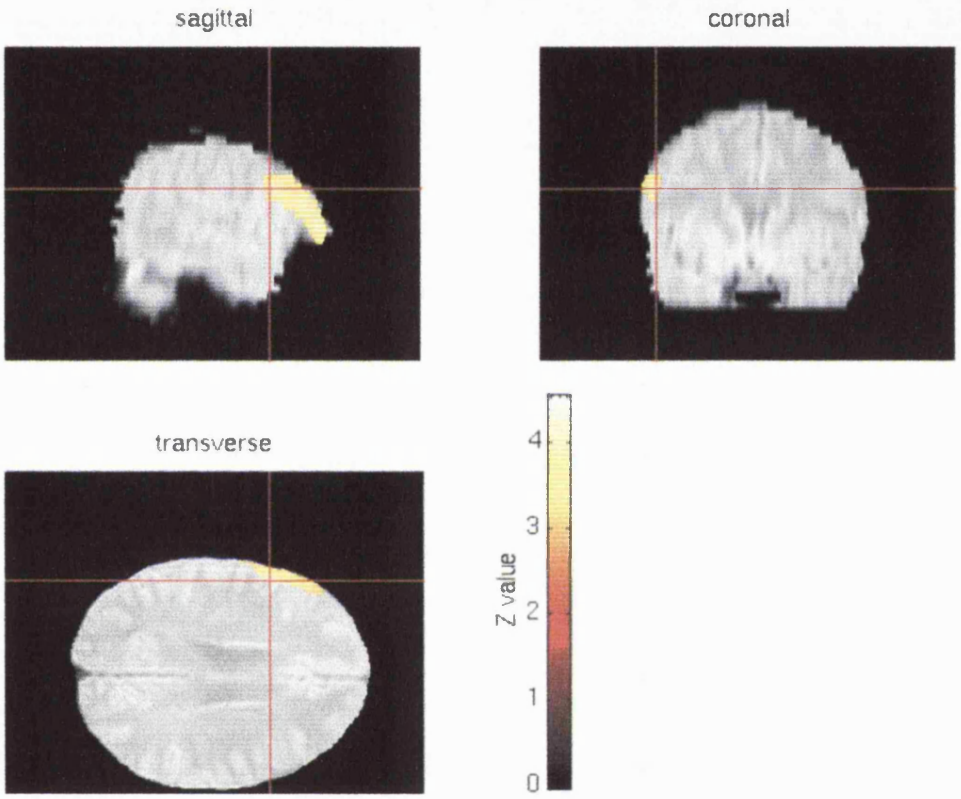
Figure 12. MTR reductions in temporal regions



Group differences in structural MRI

The same levels of smoothing and grey matter threshold used for the MTR data were applied to the proton density images. Group analysis of the proton density weighted images in SPM revealed some areas of significantly reduced signal intensity, suggestive of cortical volume loss or atrophy, in the left inferior frontal cortex in the schizophrenic group (Figure 13). These areas were far less extensive than the cortical areas of reduced MTR. This suggests that the MTR changes may reflect subtle or early structural abnormalities that may be present prior to detection on conventional MRI.

Figure 13. Group differences in signal intensity on proton density maps



P values & statistics:

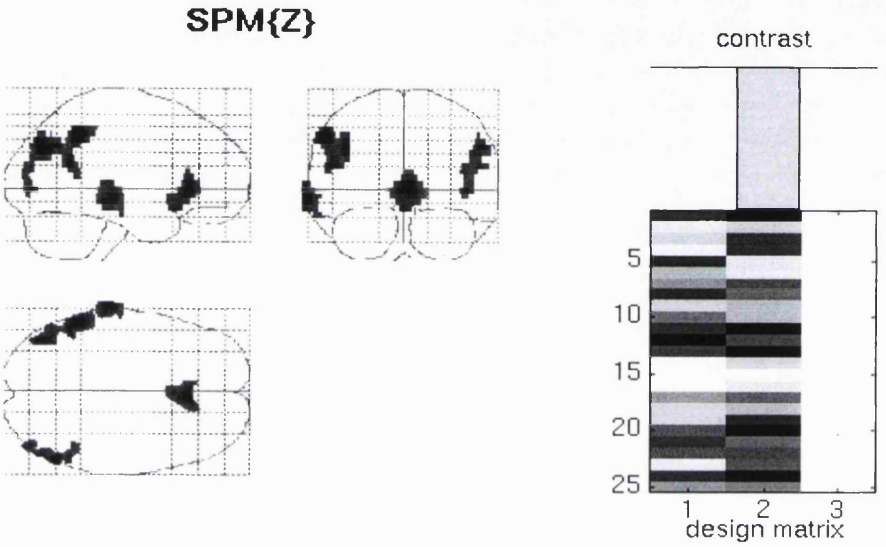
set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z	x,y,z {mm}
0.001 (2)	**0.002 (2146, 4.57)**	**0.035 (4.57)**	**0.000**	**0.000**	**-55 13 25**
		0.177 (4.13)		0.000	-54 34 15
		0.200 (4.09)		0.000	-56 40 5

Relationship between MTR and clinical variables

Covariate analysis using SPM was also performed on the MTR data. Clinical variables of age, duration of illness, current dose of antipsychotic medication and PANSS scores were used as covariates to determine whether MTR changes were related to these variables and whether these correlations were localised to specific brain regions.

There were no significant MTR reductions with increasing age in either the patient or control group although there was a trend for the changes being more widespread in the patients compared to controls prior to correction for multiple comparisons. In the schizophrenic group, duration of illness and current dose of antipsychotic medication (chlorpromazine dose equivalents) were not related to MTR. Areas of significant MTR reductions at the cluster level in bilateral temporo-occipital cortex, left inferior parietal and the genu of the corpus callosum were observed with increasing severity of negative but not positive symptoms suggesting that focal MTR changes may be related to the severity of particular clinical subtypes (Figure 14). At the voxel level, the left parietal and left temporo-occipital areas were still significantly related to the severity of negative symptoms and there was a trend towards significance for the genu of the corpus callosum and right temporo-occipital areas.

Figure 14. Correlation between MTR changes and severity of negative symptoms



P values & statistics:

set-level {c}	cluster-level {k,Z}	voxel-level {Z}	uncorrected k & Z	x,y,z {mm}
0.000 (4)	**0.003 (1770, 4.86)**	**0.011 (4.86)**	**0.000**	**0.000**	**-42 -73 30**
		0.013 (4.82)		0.000	-56 -48 45
		0.036 (4.58)		0.000	-51 -53 20
	0.011 (1215, 4.42)	**0.066 (4.42)**	**0.001**	**0.000**	**3 30 5**
		0.337 (3.93)		0.000	1 20 -15
	0.045 (766, 4.41)	**0.069 (4.41)**	**0.006**	**0.000**	**-70 -25 -10**
		0.546 (3.74)		0.000	-71 -28 5
		0.935 (3.32)		0.000	-64 -19 -15
	0.017 (1057, 4.24)	**0.129 (4.24)**	**0.002**	**0.000**	**54 -62 35**
		0.317 (3.96)		0.000	44 -82 0
		0.503 (3.77)		0.000	53 -54 20

Height threshold {u} = 3.09, p = 0.001
Extent threshold {k} = 6.599408e+02 voxels, p = 0.010
Expected voxels per cluster, E{n} = 90.1
Expected number of clusters, E{m} = 0.1

Volume {S} = 441867 voxels or 520.4 Resels
Degrees of freedom due to error = 22.0
Smoothness {FWHM mm} = 14.6 14.8 17.3
{voxels} = 6.2 6.3 7.3

DISCUSSION

The purpose of this study was to explore and characterise cortical abnormalities in vivo in patients with schizophrenia using MTR. The results of this study suggest that there are diffuse bilateral cortical abnormalities in schizophrenia, predominantly in the fronto-temporal cortex. These abnormalities only extend into the white matter in the temporal areas. This study provides the first evidence that MTR is a useful tool to detect subtle cortical abnormalities where little cortical volume reduction is evident.

MTR abnormalities in the cortex

The predominant localization of MTR reduction to the fronto-temporal regions is in keeping with the results reported by others using conventional MRI that have suggested schizophrenia involves mainly fronto-temporal networks (Weinberger et al, 1992; Wright et al, 1999; Sigmundsson et al, 2001). The interest of this work is twofold. Firstly, it shows that MTR abnormalities are far more extensive than those detected with conventional MRI and secondly, that these abnormalities are present before detectable volumetric losses occur.

It is difficult to be specific about the nature of the changes in the cortex as the histopathological counterparts of MTR changes in grey matter have not been fully explored. As described in Chapter 3, histopathological studies in schizophrenia have reported neuronal abnormalities, namely, changes in neuronal density, number and size although the findings have been inconsistent. Further indirect support for neuronal abnormalities in schizophrenia comes from MRS findings of reduced NAA, considered to be a marker of neuronal or axonal integrity, in the temporal lobes including the hippocampus (Yurgelun-Todd et al, 1996a; Renshaw et al, 1995; Maier et al, 1996;

Lim et al, 1998) and frontal regions (Bertolino et al, 1996). It has also been suggested that the neuronal abnormalities in schizophrenia may be subtle and restricted to specific cortical layers with reports of reduced interstitial neurons in superficial compartments (Akbarian et al, 1996a) and decreased neuronal size in layer IIIc (Rajkowska et al 1998) of the prefrontal cortex.

More recent histopathological studies using stereological techniques (Pakkenberg et al, 1993; Selemon et al, 1998) have found an increased in neuronal density in the presence of cortical volume reduction. These findings have led to the suggestion that abnormalities in the neuropil, which includes neuronal processes such as dendrites and synapses, may be more significant than neuronal loss in schizophrenia or at least partly account for the cortical changes (Selemon et al, 1998; Glantz and Lewis, 2000). In addition, studies of synaptic pathology have provided further evidence of dendritic (Glantz and Lewis, 1997, Rosoklija et al, 2000; Cotter et al, 2000) and synaptic (Eastwood et al, 1995; Glantz and Lewis, 1997; Eastwood and Harrison, 1998; Young et al, 1998) abnormalities in the brain in schizophrenia. The results of this study are therefore compatible with these neuropathological findings of subtle abnormalities in the cortex involving neurons or neuronal processes in schizophrenia. It is likely that such abnormalities may disrupt the neural circuitry and result in altered connectivity. This would support the theory that schizophrenia is a disorder of brain connectivity which has mainly originated from findings of abnormal functional connectivity, particularly in frontotemporal regions, in schizophrenic patients (Frith et al, 1995; McGuire and Frith, 1996a). More recently, it has been suggested that anatomical connections are also disrupted in schizophrenia. Some studies have reported abnormal correlations between regional brain volumes in the fronto-temporal (Woodruff et al,

1997b) or thalamo-cortical (Portas et al, 1998) regions. Others have suggested that the anatomical connections are abnormal on a microscopic level in schizophrenia and that any alteration in the dendritic arbor may affect interneuronal connectivity (Selemon et al, 1999).

Clinical correlates

An intriguing finding of the study is the association of MTR reduction in the left parietal, bilateral temporo-occipital cortex and genu of the corpus callosum with negative symptoms. This would lend some support to previous reports that negative symptoms are related to focal structural abnormalities such as the corpus callosum (Gunther et al, 1991; Tibbo et al, 1998; Woodruff et al, 1997a). However, the findings may have been affected by the patients having only mild to moderate negative symptoms. Only six patients had total scores greater than 24 (midpoint of the range of total scores) and further analysis revealed a non significant trend of more widespread MTR reductions in these patients compared to those with less severe negative symptoms. Therefore the findings do not exclude the possibility that MTR changes in other regions such as the frontal lobes may also be related to more severe negative symptoms as has been suggested by others (Baare et al, 1999; Sanfilipo et al, 2000).

No association between MTR abnormalities and duration of illness for the chronic schizophrenic patients in this study was found. There is however, a suggestion that MTR abnormalities may be progressive at least in some patients from recent preliminary findings of MTR reductions in patients with first episode schizophrenia (Bagary et al, 2001). The MTR reductions in these first episode patients were less widespread than in the chronic patients but also mainly detected in the fronto-temporal

regions, namely, the insula, anteromedial frontal cortex, anterior cingulate and right superior and middle temporal gyri. Further evidence that cortical abnormalities may be progressive in schizophrenia comes from a recent MRI study in patients with severe early onset schizophrenia who were studied over a 5 year period (Thompson et al, 2001). Cortical deficits in these patients were initially detected in parietal regions and later extended to the temporal and dorsolateral prefrontal regions. Only longitudinal studies can determine whether MTR abnormalities are progressive in schizophrenia.

The patients in this study had been on antipsychotic medication for many years but it seems unlikely that the effect of antipsychotic medication was a significant confounding factor. Although an unreliable measure, no association between MTR abnormalities and the current dose of medication was observed. In addition, there is little evidence accruing from neuropathological studies that cortical abnormalities can be attributed to antipsychotic medication (Harrison,1999).

In this study, no significant age-related MTR changes in either group was detected in contrast to previous reports of subtle age-related MTR reductions in white matter in a healthy population using a ROI methodology (Silver et al, 1997). This may have been due to the narrower age range of the subjects compared to the other study.

Methodological considerations

Voxel-based analysis has been applied to both functional and structural imaging data. More recently, it has been used in DTI studies but has not previously been applied to MTR data. A voxel-based approach provides an objective technique that allows the whole brain to be analysed avoiding the subjective bias in the selection of regions of

interest and in determining structural and tissue boundaries. This allows the differences in the local composition of brain tissue to be examined after macroscopic differences in brain shape have been discounted. Images are compared on a voxel basis after deformation fields have been used to spatially normalise the images (i.e. mapping of the individual brain onto a standard reference brain). A number of software packages have been designed for voxel-based analysis.

The voxel-based automated analysis, SPM96, was used to examine group MTR differences in grey and white matter in this study. This is the first time that a voxel-based analysis has been used for MTR data. This technique allowed a global analysis to be performed and avoided the *a priori* bias of using a ROI approach. In this study, it would have been difficult to avoid partial volume effects in selecting regions of interest in the cortical grey matter areas identified from the voxel-based analysis.

For the SPM statistics in this study, the cluster level was used rather than the voxel level of significance as histograms of a sample of the data incorporating white and grey matter on a given slice resembled a normal distribution. This satisfies the recently suggested criteria for the use of cluster size to assess significance in SPM which is only considered appropriate if the data are normally distributed (Ashburner and Friston, 2000). Furthermore, the lack of significant MTR changes between the two groups of controls, using the same level of smoothing, grey matter threshold and level of significance, suggests that the differences in MTR between schizophrenics and controls in this study are likely to be biologically significant.

Normalization of the images was performed to increase the sensitivity for detecting focal changes by reducing the anatomical variability between subjects and by using a reliable segmentation process, it was possible to separate grey and white matter abnormalities. The temporal white matter MTR reductions in the schizophrenic patients in this study confirm the findings reported in Study 1 using a ROI analysis. These temporal white matter MTR changes are likely to reflect a focal disruption of axonal or myelin integrity. This would result in a disruption of axonal connections and thus provides further support for the theory of altered anatomical connectivity in schizophrenia. The finding of focal white matter abnormalities is in contrast to previous reports of a widespread reduction of anisotropy in white matter (Lim et al, 1999) or prefrontal white matter (Buchsbaum et al, 1998) using DTI. One possible reason for this is that the MTR changes in other areas of white matter may be more subtle than in the temporal lobes and were not detected with this technique and sample size. Therefore, despite the sensitivity of the technique, the results do not exclude the possibility of more subtle abnormalities in other regions of white matter.

Several limitations to this study should be considered. The sample size in this study was small and it may not be possible to generalise the findings to other schizophrenic populations. Gender differences in MTR were not examined in this study, as there were considerably fewer females than males in both groups. A recent meta-analysis found little evidence of gender differences in regional and global cerebral volumes in schizophrenia (Wright et al, 2000) but less is known about gender differences in the histopathological changes. It is of interest that recent studies in normal subjects, albeit in small samples, have reported that males have greater neuronal density than females and reciprocally, females have increased neuropil in the presence of similar cortical

thickness (de Courten-Myers, 1999; Rabinowicz et al, 1999). This has led to speculation that this may explain the greater incidence of Alzheimer's disease, characterised by neuronal loss, in females. Therefore, it is possible that this may also explain the greater incidence of schizophrenia in males if schizophrenia proves to be a disease involving the neuropil of which females have a greater reserve. A further limitation to the study was the lack of 3-dimensional volume MRI data sets acquired at the time of the study which would have been more accurate in determining cerebral volume reduction than the proton density images used. The proton density images were selected in preference to T2 weighted images as they were considered to provide clearer discrimination of tissue from the CSF. It is unlikely that the MTR reductions in the patients were solely related to atrophy as more widespread changes on the proton density images would have been expected. However, it is possible that the true extent of cerebral volume reduction may not have been detected and may have partly contributed to some of the findings.

Longitudinal studies are needed to establish whether MTR abnormalities are present at the onset of the illness and whether they are progressive, how they may relate to other disease manifestations such as cognitive changes and their prognostic significance.

CONCLUSIONS

Given the methodological limitations of post mortem studies, the introduction of novel neuroimaging techniques, namely, MTI and DTI, have enabled further exploration and characterization of the neuropathological abnormalities in vivo in patients with schizophrenia. The findings from the three interlinked studies in this thesis confirm that MTI and DTI are capable of providing more neuropathological information in vivo in schizophrenia than conventional MRI and may be more sensitive to subtle or early neuropathological changes. The main findings from this thesis are summarised below:

1. White matter abnormalities

White matter abnormalities were detected in this group of schizophrenic patients using MTI and DTI but were only restricted to bilateral temporal lobes and not widespread. In particular, the studies failed to detect any abnormalities in frontal white matter. The bilateral temporal white matter abnormalities are likely to reflect a focal but subtle disruption of axonal or myelin integrity. In addition, the presence of DTI changes in the splenium but not the genu of the corpus callosum suggest that there may be a focal disruption of commisural connectivity in schizophrenia involving interhemispheric fibres from the inferotemporal fibres and not fibres from the frontal lobes. Thus, these findings do not support one of the hypothesis of this thesis that white matter abnormalities would be detected in the fronto-temporal regions or that they are the underlying neuropathological changes of abnormal connectivity in schizophrenia.

2. Cortical abnormalities

Widespread cortical abnormalities were detected in the same group of patients using MTR. The MTR changes were predominantly in the fronto-temporal regions and with the exception of the temporal lobes, these changes did not extend into the white matter. This is in keeping with previous structural imaging studies that have mostly reported grey matter and not white matter abnormalities in chronic schizophrenia. The MTR abnormalities in the cortex were unrelated to volume reduction and may reflect subtle neuropathological changes involving the neuropil. These findings are compatible with the hypothesis of disrupted fronto-temporal neural circuitry in schizophrenia.

3. Clinico-pathological correlates

The relationship between the neuroimaging findings and clinical variables was also explored in all three studies. Age related changes in MTR were not demonstrated in these studies although this may have been due to the narrow age range and the fact that subtle age related changes in MTR have been mainly reported in older subjects. There was little evidence that MTR or DTI abnormalities were related to duration of illness but only longitudinal studies can determine whether these abnormalities are progressive during the course of the illness. The lack of correlation between MTR or DTI changes with dose of antipsychotic medication was not surprising given that structural changes that have been reported to be associated with antipsychotic medications in previous neuroimaging studies have been mainly in the basal ganglia. The relationship between clinical symptomatology and MTR or DTI findings was also examined as there has been some previous suggestion that schizophrenic symptomatology can be related to structural abnormalities. However, the findings in these studies were only modest and it was only in Study 3 that the severity of negative

but not positive symptoms was found to be associated with MTR reductions in particular areas, such as bilateral parieto-occipital cortex and the genu of the corpus callosum. This may have been due to sample bias as patients in these studies only had mild to moderate negative and positive schizophrenic symptoms.

4. Limitations to the studies

A number of reasons may be put forward to explain the discrepancies between the MTR and DTI findings in these studies. It is possible that MTR may be a more sensitive technique than DTI in detecting subtle changes. These techniques may also be measuring different pathological processes which may have different effects on MTR and DTI measures. MT changes rely on the transient binding of water molecules to macromolecules whereas diffusion is affected by the boundaries to the motion of water. MTR changes reflect the ability of the matrix molecules to exchange energy with the surrounding water and therefore provide an indirect measure of the integrity of the structural matrix which contains myelin and other cell membranes whereas changes in diffusion measures are considered to be more specifically related to axonal integrity. The lack of correlation between the two measures has also been demonstrated in a study of MS patients which compared average MTR and mean diffusivity on histograms suggesting that this may be due to the complex relationship between the different pathological mechanisms and their effect on MTR and DTI measures (Cercignani et al, 2001a). Furthermore, it is important to consider that methodological issues concerning data acquisition or analysis, particularly for DTI, may have contributed to the findings in this thesis.

The stringent research methodology that is needed to ensure that results can be generalised is more likely to be met by neuroimaging than post mortem studies. However, it is only with the standardisation of image acquisition and methods of image analysis capable of taking individual variation into account and avoiding multiple comparisons, that meaningful comparisons can be made between different studies. The use of region of interest and voxel-based analyses for MTI and DTI data also needs to be further evaluated. It remains to be determined if improved data acquisition and analysis for DTI will further elucidate the changes in white matter fibre tracts in schizophrenia.

Another limitation to the studies in this thesis was the small sample sizes and therefore further studies are needed to confirm these findings. Furthermore, future studies applying MTI and DTI to other major psychiatric illnesses such as bipolar disorder are needed to determine whether these findings are specific to schizophrenia. Comparative studies that include other disorders in which schizophrenia-like symptoms may occur such as in temporal lobe epilepsy, may also shed light on the neuropathology of schizophrenia or psychosis in general. The majority of structural imaging studies are cross-sectional and it is difficult to know for certain if there is any progression during the courses of the illness. Thus, longitudinal studies are essential to determine whether these subtle neuropathological abnormalities detected with MTR or DTI are present early in the illness and whether they progress.

Summary

The results from this thesis suggest that detectable pathology in schizophrenia is predominantly cortical and preferentially in the fronto-temporal regions. This suggests

that abnormal connectivity in schizophrenia is more likely to be related to cortical changes without gross disruption to the white matter tracts. These studies also illustrate the potential of using novel neuroimaging techniques, namely MTI and DTI, to investigate the neuropathology in schizophrenia although methodological issues relating to their data acquisition and analysis should be carefully considered.

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APPENDIX 1. PROTOCOL FOR SELECTING REGIONS OF INTEREST (ROIs) IN WHITE MATTER

Frontal

Identify two landmarks in the frontal lobe:

a. the first slice in which grey matter is prominent with only a restricted amount of white matter.
b. the first slice in which partial volume of the ventricles can be seen.

Select the **middle slice** between these two landmarks to place the ROI in white matter. Avoid ventricles and grey matter gyri (partial volume effects).

Parietal

In the slices in which the insula can be identified, the middle slice will be considered the junction between frontal and temporal and frontal and parietal lobes.

Superior to this slice, select the slice in which the intraparietal sulcus can be identified (it is surrounded by grey matter). The anterior border is the central sulcus. Place the ROI in the centre of this white matter area.

Occipital

Identify the slice in which the cerebellum is first seen.

Select the first slice superior to this one where there is sufficient white matter. Place the ROI posterior to the parieto-occipital sulcus.

Temporal

Identify two landmarks:

a. the most inferior slice from the skull base

b. the middle slice in which the insula is seen.

Moving up from the most inferior slice, select the slice in which the margins of the temporal lobe are still seen. Place the ROI in the white matter anterior to the occipital lobe margin and lateral to the temporal horn of the lateral ventricle.

Corpus callosum (splenium)

Select the slice in which the trigones of the lateral ventricles are seen and posterior margin of the splenium itself is against the termination of the interhemispheric fissure and its adjacent grey matter. Place the ROI in the midline behind the third ventricle.

APPENDIX 2. PUBLICATIONS FROM THIS THESIS

1. **An in vivo investigation of white matter pathology in schizophrenia using magnetization transfer imaging.**

   J Foong, M Maier, GJ Barker, S Brocklehurst, DH Miller and MA Ron

   (JNNP 2000; 68: 70-74)

2. **Neuropathological abnormalities of the corpus callosum in schizophrenia: A diffusion tensor imaging study.**

   J Foong, M Maier, CA Clark, GJ Barker, DH Miller and MA Ron

   (JNNP 2000; 68: 242-244)

3. **Neuropathological abnormalities in schizophrenia: Evidence from magnetization transfer imaging.**

   J Foong, MR Symms, GJ Barker, M Maier, FG Woermann, DH Miller and MA Ron

   (Brain 2001; 124:882-892)

4. **Investigating regional white matter in schizophrenia using diffusion tensor imaging.**

   J Foong, MR Symms, GJ Barker, M Maier, DH Miller and MA Ron

   (Neuroreport 2002, in press)