CHAPTER 1

GENERAL INTRODUCTION

1.1 PREAMBLE

Schizophrenia is one of the most devastating diseases to have ever affected humanity. Elucidating the origins and nature of the disease is one of the most important challenges facing modern medical science, as it underpins the extent to which the disease can be successfully treated. Although schizophrenia has long been recognized as being caused by a neurological abnormality, the specific structural brain defects underlying the disease remain unclear. The primary aim of this thesis was to use the neuroimaging technique of Magnetic Resonance Imaging (MRI) to identify the neuroanatomical underpinnings of schizophrenia in patients experiencing their first psychotic episode, and to elucidate whether these neuroanatomical abnormalities were static or progressive over the first 2-3 years of illness. Furthermore, this thesis also aimed at identifying the clinical and electrophysiological correlates of the neuroanatomical abnormalities present in patients with first-episode schizophrenia, in the hope that this might provide some further insight into the nature of the functional abnormalities characteristic of the disease.

The aim of this chapter was to describe the clinical profile of schizophrenia, outline the pre-eminent psychosocial and neurobiological conceptualisations of schizophrenia that have been proposed in the literature, describe the methods that have been used to identify the neuroanatomical underpinnings of the disease, and to provide an overview of the previous studies that have used MRI to elucidate these neuroanatomical underpinnings in both chronic and first-episode patients with schizophrenia. I have begun this chapter by

considering the enormous personal, social and economic costs associated with the disease of schizophrenia.

1.2 THE COST OF SCHIZOPHRENIA

Schizophrenia is a severe mental illness that affects approximately 1% of the population worldwide, or approximately 60 million people (Jablensky, 1997). It is a debilitating and stigmatising illness that generally has a devastating effect on the lives of its sufferers and their families. Schizophrenia has a typical age-of-onset in late adolescence or early adulthood, and its influence is so destructive that it has been referred to as "*youth's greatest disabler*" (Ashe, Berry, & Boulton, 2001). Subsequent to the onset of schizophrenia, the majority of sufferers remain affected for the rest of their lives. A substantial proportion of patients (approximately 20%) require lifelong care or hospitalisation, which places an enormous burden on health care systems (Lang, Forbes, Murray, & Johnstone, 1997). In a study undertaken in 2003, it was estimated that the economic cost of schizophrenia in Australia was approximately $600 million per annum in health care costs alone (Carr, Neil, Halpin, Holmes, & Lewin, 2003), or approximately 2% of the total governmental expenditure on health and community services (Carr, Lewin, Neil, Halpin, & Holmes, 2004). Sufferers of schizophrenia are unlikely to obtain or maintain steady employment (Sevy & Davidson, 1995), have poorer physical health (Osborn, 2001; McCreadie, 2003) and far higher rates of substance abuse than the general population (Green, Salomon, Brenner, & Rawlins, 2002), and are far more likely to become homeless or to live in poverty (Herman, Susser, Jandorf, Lavelle, & Bromet,

1998; Craig & Timms, 1995). Furthermore, sufferers of schizophrenia are considerably less likely than average to get married (Thara & Srinivasan, 1997) or have children (Nimgaonkar, 1998), and are much more likely to experience depression, with a rate of depression estimated at 25% (Siris, 1995). It has been estimated that 10% of people with schizophrenia end up committing suicide, which is a rate nine times higher than the general population (Harris & Barraclough, 1997). Although schizophrenia is not a fatal disease, notwithstanding the exorbitant suicide rate, the suffering that it produces and its cost to society is so great that an editorial in the journal Nature described schizophrenia as "*arguably the worst disease affecting mankind, even AIDS not excepted*" (Nature Editorial, 1988 Vol. 336, p.95).

1.3 THE DIAGNOSTIC ENTITY OF SCHIZOPHRENIA

The term 'schizophrenia' was originally coined by the German psychiatrist Eugen Bleuler (Bleuler, 1911) to describe what he thought was the defining symptom of the disease: a loosening of the mental associations between cognitions. Schizophrenia is derived from the Greek words *phrene,* meaning mind, and *schizo,* which has been traditionally translated as 'split', but in light of Bleuler's intentions is perhaps better translated as 'fragmented', as suggested by Andreasen (1999). Indeed the extremely common misinterpretation of schizophrenia as referring to the very rare condition of multiple personality disorder is one of the most pervasive inaccuracies in the history of medicine, and has led some commentators to suggest that 'schizophrenia', as a diagnostic

term, is misleading and in need of an overhaul (Duckworth, Halpern, Schutt, & Gillespie, 2003).

Schizophrenia has an extremely heterogeneous clinical profile, which is to say that the clinical symptoms can vary markedly from patient to patient. There is no 'essential' symptom that is necessary or sufficient for a person to be diagnosed as suffering from schizophrenia. Indeed, Andreasen et al. (1999) have commented that two patients can be diagnosed as both suffering from schizophrenia despite not having a single symptom in common. In spite of this, however, there are a number of symptoms that are typically associated with schizophrenia which, given their idiosyncrasy, makes schizophrenia one of the more clinically distinctive mental illnesses.

The symptoms of schizophrenia have traditionally been divided into 'positive symptoms' and 'negative symptoms' (Cutting, 1995). Positive symptoms refer to phenomena that are experienced by sufferers of schizophrenia that are not experienced by healthy individuals, while negative symptoms refer to experiences or behaviours that are typically present in healthy people, but which are absent in patients with schizophrenia.

1.3.1 The 'positive' symptoms of schizophrenia

The common 'positive' symptoms of schizophrenia are hallucinations, delusions and formal thought disorder.

Hallucinations are false sensory perceptions that the patient experiences as being real. They may occur in any sensory modality (i.e. auditory, visual, olfactory, gustatory or tactile), however auditory hallucinations are by far the most common in schizophrenia, and are usually experienced as 'hearing voices'. These voices, which can be either familiar or unfamiliar to the patient, are perceived as being externally generated (i.e. distinct from their own thoughts), although they can be perceived as occurring either inside or outside their own heads. Voices maintaining a running commentary on the patient's thoughts or behaviour, such as those described in the example below, are considered especially characteristic of schizophrenia. "*She is peeling potatoes, got hold of the peeler, she does not want that potato, she is putting it back, because she thinks it has a knobble like a penis, she has a dirty mind, she is peeling potatoes, now she is washing them*" (from Mellor, 1970, p.16).

Hearing two or more voices arguing or maintaining a conversation is another type of auditory hallucination that is particularly common in schizophrenia. For example: *"A twenty-four-year-old male patient reported hearing voices coming from the nurse's office. One voice, deep in pitch and roughly spoken, repeatedly said 'G.T. is a bloody paradox', and another higher in pitch said 'He is that, he should be locked up'. A female voice occasionally interrupted saying, 'He is not, he is a lovely man'"* (from Mellor, 1970, p.16).

The voices are most often disparaging or threatening and can sometimes, in the case of 'command hallucinations' instruct the sufferer to perform a particular action that s/he

finds deeply abhorrent. For example: *"Kill yourself. Chop up your girlfriend"* (from Close & Garety, 1998, p.179).

Delusions are strongly held, false beliefs that are maintained in spite of overwhelming evidence to the contrary. Persecutory delusions are very common in schizophrenia, in which the patient believes that s/he is being conspired against, spied on, followed, mocked or somehow persecuted. For example: *"People at work are victimizing me. A bloke at work is trying to kill me with some kind of hypnosis"* (from Frith, 1992, p.66). Delusions of grandeur also occur in schizophrenia, although they are less common than persecutory delusions, and refer to the patient's belief that they are particularly important or influential or have magical powers. For example, the patient may believe that they are Jesus Christ. Delusions of reference are also common and refer to patients' belief that passages from books or newspapers, radio commentaries, other people's apparently innocuous comments, etcetera, have special significance to them. For example: *"I saw someone scratching his chin which meant that I needed a shave"* (from Frith, 1992, p.66).

Other common delusions in schizophrenia include the delusion of thought insertion, in which the sufferer believes that some of his thoughts are not his own, but have been inserted by some external agent. For example: *"Thoughts are put into my mind like 'Kill God'. It's just like my mind is working but it isn't. They come from this chap Chris. They're his thoughts"* (from Frith, 1992, p.66). The converse delusion of thought withdrawal is also common. For example: *"I am thinking about my mother, and suddenly*

the thoughts are sucked out of my mind by a phrenological vacuum extractor, and there is nothing in my mind, it is empty" (from Mellor, 1970, p.16). Delusions of alien control, in which the patient believes that his actions are not self-generated but are being controlled by some external agent, are also common. For example: *"They inserted a computer into my brain. It makes me turn to the left or right"* (from Mellor, 1970, p.16).

Formal thought disorder (otherwise known as 'disorganized thought'), is characterized by a loosening of the mental associations between cognitions, and has been argued by Bleuler (1911) and others (Andreasen, 1999) to be the single most important symptom in schizophrenia, and the one which underlies the more florid symptoms of psychosis. Formal thought disorder is most often inferred through the characteristic abnormalities of spoken language exhibited by patients with schizophrenia (Cutting, 1995). Thought-disordered patients tend to jump from one topic to the next in conversation, with the links between the topics being unclear or illogical – a phenomenon known as 'derailment'. For example: *"What I'm saying is my mother is too ill. No money. It all comes out of her pocket. My flat's leaking. It's ruined my mattress. It's Lambeth council. I'd like to know what the caption in the motto under their coat of arms is. It's in Latin"* (from Cutting, 1985). The relevance of thought-disordered patients' answers to questioning is often unclear or completely unrelated – a phenomenon known as 'tangentality'. For example: *"Q: What city are you from? A: 'I was born in Iowa, but I know that I'm white instead of black so apparently I came from the south somewhere and I don't know where, you know, I really don't know where my ancestors came from"* (from Andreasen, 1979). The speech of thought-disordered patients is sometimes so disorganized as to be incoherent. The

presence of thought disorder is sometimes inferred not from abnormalities in patients' spoken language, but from abnormalities in their overt behaviour. For example, thought disorder may be diagnosed if the patient is dressed unusually (e.g. wearing multiple overcoats), displays inappropriate sexual behaviour (e.g. public masturbation), or unpredictable agitation (e.g. shouting or swearing) (American Psychiatric Association, 1994).

1.3.2 The 'negative' symptoms of schizophrenia

In contrast to the 'positive' symptoms, the 'negative' symptoms of schizophrenia refer to experiences or behaviours that are typically present in healthy subjects but that are absent in people with schizophrenia. The three most common 'negative' symptoms in schizophrenia are affective flattening, alogia and avolition. These three symptoms are the only 'negative' symptoms that are included as diagnostic criteria for schizophrenia in DSM-IV (American Psychiatric Association, 1994). Affective flattening is especially common in schizophrenia, and is characterized by a reduced ability to express emotion. The faces of patients with affective flattening often appear immobile and unresponsive, and show a 'mask-like' expression. Patients with affective flattening also typically exhibit reduced levels of eye-contact, body movement and non-verbal communication (e.g. hand gestures). Patients with alogia (poverty of speech) typically do not initiate conversation, and reply when questioned with overly brief, laconic answers often devoid of content. Avolition (poverty of will) is characterized by an inability to initiate and persist with goal-directed activities. Patients with avolition are easily distracted from the

task at hand, and often show little interest in participating in work or recreational activities.

The ‘negative’ symptoms of schizophrenia are more ubiquitous than the ‘positive’ symptoms, with most of them occurring in other mental disorders, most notably depression. This may not be coincidental, as it has been suggested that ‘negative’ symptoms and symptoms of depression share the same underlying mechanism, involving a dysfunction in the reward systems of the brain (Schmidt et al., 2001).

The positive/negative symptom dichotomy has remained popular in the literature, and has formed the theoretical basis for two of the most influential clinical rating scales used in schizophrenia – the Positive and Negative Syndrome Scale (PANSS) (Kay, Opler, & Lindenmayer, 1989) and the Scale for the Assessment of Positive (SAPS) / Negative (SANS) symptoms (Andreasen, 1984a; Andreasen, 1984b). In recent years, however, factor analytic studies have led to the development of a tripartite model of schizophrenic symptomatology. In the tripartite model, the ‘positive’ symptoms are separated into two separate factors, while the ‘negative’ symptoms constitute the third factor (Liddle, 1987b). The first factor in the tripartite model is the Reality Distortion factor (which has also been called the Psychosis factor (Andreasen, Arndt, Alliger, Miller, & Flaum, 1995), and it encompasses the symptoms of hallucinations and delusions. The second factor is the Disorganization factor, and it encompasses the symptoms of disorganized thought, disorganized behaviour and inappropriate affect. The third factor, Psychomotor Poverty, encompasses the remaining ‘negative’ symptoms.

In spite of the rise of the tripartite model, however, the positive/negative symptom dichotomy remains the most popular framework for classifying the clinical manifestations of schizophrenia, and remains the theoretical underpinning for most of the major theories of schizophrenia.

I will now consider some of the theories, both past and present, which have speculated as to the nature of schizophrenia, and the causes underlying it.

1.4 PSYCHOSOCIAL AND ENVIRONMENTAL THEORIES OF SCHIZOPHRENIA

Most of the early models of schizophrenia emphasized the role of psychological, social and environmental factors in the development of the disease.

The first psychoanalytic theory of schizophrenia was proposed in the 1920s by Harry Stack Sullivan and, like all psychoanalytic theories, emphasised the role of psychological conflicts between the three strata of consciousness (i.e. id, ego and superego) in the development of the disease. Sullivan (1924) argued that the symptoms of schizophrenia reflected a regression to early childhood forms of communication arising from a failure to cope with the societal pressures due to the patient's fragile ego. Another psychoanalytic theory, coined the 'schizophrenogenic mother hypothesis', became very popular in the 1950s and 60s and emphasized the role of early childhood relationships in the

development of schizophrenia. The hypothesis was first postulated by Frieda Fromm-Reichmann (1948), but was subsequently adopted and revised by a number of other theorists (see Hartwell, 1996 for a review). The 'schizophrenogenic mother hypothesis' argued that schizophrenia was initiated in childhood by a cold, domineering, ambitious mother who was resentful of her role as a housewife and who thwarted the child's attempts at independence as a means by which she could acquire some consolatory power. John Rosen summarized the 'schizophrenogenic mother hypothesis' in the opening line of a paper entitled *"The Perverse Mother": "a schizophrenic is always one who is reared by a woman who suffers from a perversion of the maternal instinct"* (Rosen, 1953, p.97). The 'schizophrenogenic mother' hypothesis persisted until the 1970s in spite of a growing body of empirical evidence which indicated that there were minimal differences in the parenting styles of the mothers of children with schizophrenia, and the mothers of children without schizophrenia (e.g. Ringuette & Kennedy, 1966).

Another theory that emphasized the role of childhood environment in the development of schizophrenia was the 'sociogenic hypothesis' (e.g. Garmezy & Streitman, 1974). The sociogenic hypothesis arose in response to evidence suggesting that the rate of schizophrenia was disproportionately high in the lower socio-economic classes (e.g. Srole, Langner, Michael, Opler, & Rennie, 1962). Proponents of the sociogenic hypothesis argued that this relationship was causal, that is, the stressors associated with being of low socio-economic class (e.g. low levels of education, living in a violent community, lack of opportunity, poor nutrition etc.) were the direct cause of schizophrenia. More recent studies have focused on the role of the biological stressors

associated with low socio-economic status rather than the social stressors *per se*. For example, Susser et al. (1996) found that the children of mothers whose nutrition was poor during pregnancy were at an increased risk of developing schizophrenia.

One final and controversial theory that emphasized the role of society and the environment in the development of schizophrenia was the 'labelling theory'. The labelling theory, originally proposed by Scheff (1966), argued that schizophrenia was not a disease as such, but rather a learned societal role. According to this model, healthy people often performed behaviours that could be classified as being 'socially deviant', and that these behaviours could lead to them being diagnosed as suffering from a mental illness. For example, a person laughing at a funeral could be diagnosed as suffering from formal thought disorder and thus schizophrenia. According to this model, once people were labelled as being mentally ill they began to act in a way consistent with the label. For example, by being labelled as suffering from schizophrenia, a healthy person would begin to perceive themselves as suffering from schizophrenia and would thus begin to perform actions consistent with being a patient with schizophrenia. Given more recent evidence regarding the considerable brain abnormalities associated with schizophrenia (Section 1.6.6 – 1.6.7), this theory is unlikely to be correct or at least provide the full story regarding the onset of schizophrenia. Furthermore, it seems unlikely that patients with schizophrenia are simply acting out a learned role, given the severity and duration of their symptoms. As the psychiatrist Paul Meehl recalled after a student suggested the labelling theory to him: *"I was thinking of a patient I had seen on a ward who kept his finger up his ass 'to keep the thoughts from running out' while with the other hand he*

tried to tear out his hair because it really 'belonged to his father'. And here was this man telling me that he was doing these things because someone had called him a schizophrenic. What could I say to him?" (cited in Davison & Neale, 1998, p.275).

Undoubtedly the most significant development in the treatment of schizophrenia was the advent of antipsychotic medications in the 1950s. As has been so often the case throughout the history of medicine, the first antipsychotic drug, chlorpromazine, was developed by accident. Chlorpromazine was designed by the French chemist Paul Charpentier for use as a tranquilliser for patients undergoing surgery, and was administered to a psychotic patient Bernard P by his psychiatrist Pierre Hamon on something of a whim. Nevertheless, Bernard P's condition improved substantially with the administration of chlorpromazine, and the first antipsychotic was born. Antipsychotic medication became widely used in Europe and the USA in the mid to late 1950s, but it wasn't until the late 1960s that it was proposed that its clinical efficacy stemmed from its action as a dopamine antagonist (Kapur & Mamo, 2003).

This transformation in the treatment of schizophrenia resulted in a corresponding change in the way in which the disease itself was conceptualised. Rather than emphasising social, environmental and psychological factors as the cause of schizophrenia, theorists began to focus more on the biological underpinnings of the disease. The significance of this paradigm shift cannot be overstated, as it was at this time that schizophrenia really began to be considered primarily as a disorder of the *brain*, as opposed to a disorder of the psyche. This is not to say that the social, environmental and psychological theories of

schizophrenia are necessarily outdated or inconsistent with the 'biological theories'. In fact, each of the 'biological theories' of schizophrenia described in the section below employ a 'diathesis-stress' model of the disease. In general, the diathesis-stress model argues that people with a pre-disposition to the disease (e.g. a genetic abnormality), or a *diathesis*, will go on to develop the disease only if they are exposed to specific catalytic environmental stressors (e.g. poor nutrition in childhood). The fact that the onset of schizophrenia is more often than not associated with a major traumatic life event in adolescence (e.g. the death of a relative, break-up of a relationship, preparation for school exams etc.) is a testament to the validity of the general diathesis-stress concept (Lukoff, Snyder, Ventura, & Nuechterlein, 1984; Birley & Brown, 1970).

1.5 NEUROBIOLOGICAL THEORIES OF SCHIZOPHRENIA

1.5.1 The dopamine hypothesis

Given its enormous influence in the history of schizophrenia, it is fitting that the first biological theory of the disease to be discussed is this section is the 'dopamine hypothesis'.

An elementary form of the dopamine hypothesis was first proposed by Van Rossum in 1967: *"When the hypothesis of dopamine blockage by neuroleptic agents can be further substantiated, it may have fargoing consequences for the pathophysiology of schizophrenia. Overstimulation of dopamine receptors could be part of the aetiology…"*

(cited in Van Rossum, 2006). The subsequent discovery of the D2 dopamine receptor (Seeman, Chau-Wong, Tedesco, & Wong, 1975) led to the repeated confirmation of it being the primary site of action for all antipsychotic medications (Seeman, Lee, Chau-Wong, & Wong, 1976). Furthermore, more recent studies have reported increased synaptic dopamine and increased numbers of post-synaptic D2 receptors in patients with schizophrenia *in vivo* (Abi-Dargham et al., 2000). Finally, the administration of dopamine agonists (e.g. apomorphine) have been shown to induce psychotic symptoms in patients with Parkinson's disease (Friedman & Factor, 2000) and to worsen psychotic symptoms in patients with schizophrenia (Lieberman et al., 1984).

However, in spite of dopamine's undoubted role in the aetiology of schizophrenia, there are a number of shortcomings with the dopamine hypothesis as it was originally formulated. The first shortcoming is the fact that while antipsychotic drugs are effective in relieving the 'positive symptoms' of the disease (e.g. hallucinations, delusions), they are comparatively ineffective against the 'negative symptoms'. This suggests that there are different neurochemical mechanisms underlying the 'positive' and 'negative' symptoms of schizophrenia (Davis, Kahn, Ko, & Davidson, 1991). In support of this, while numerous studies have found evidence for increased synaptic dopamine (Seeman & Kapur, 2000) and increased numbers of dopamine receptors (Seeman, 1992) in the striata of patients with schizophrenia, other studies have suggested that the prefrontal cortex (PFC) is hypodopaminergic in patients with schizophrenia compared to healthy controls. For example, in a Positron Emission Tomography (PET) study, Okubo et al. (1997) injected a dopamine-selective radioligand into the brains of 17 drug-naïve patients with

schizophrenia and observed a reduction in the number of D1 dopamine receptors in the PFC relative to 18 matched healthy controls. Additionally, in an immunocytochemical study, Akil et al. (1999) used antibodies to tag the axons of cells producing tyrosine hydroxylase (an enzyme involved in dopamine synthesis), and reported a decrease in the density of these axons in the PFC of 16 schizophrenic subjects relative to 16 matched healthy controls, *post mortem*.

A second challenge faced by the original dopamine hypothesis comes from previous studies that have reported an association between *decreased* levels of PFC dopamine and an increase in the severity of 'negative' symptoms in patients with schizophrenia. For example, a study investigating the concentration of homovanillic acid (HVA) – which has been associated with dopamine in the central nervous system (CNS), as it is increased by the dopamine antagonist haloperidol, and decreased by the dopamine agonist apomorphine (Kendler, Heninger, & Roth, 1981; Kendler, Heninger, & Roth, 1982) – reported a positive correlation between blood-plasma concentrations of HVA and the severity of negative symptoms, as assessed by the Clinical Global Impression rating scale (Guy, 1976) in 14 male patients with schizophrenia (Davidson & Davis, 1988). Furthermore, in a PET study, Dolan et al. (1995) found that the dopamine agonist apomorphine ameliorated the impaired response of the anterior cingulate (a constituent structure of the PFC) to a cognitive task involving verbal fluency in patients with schizophrenia. Finally, the previously cited study by Okubo et al. (1997) reported that reduced numbers of dopamine receptors in the PFC was associated with an increased severity of 'negative' symptoms (assessed with the Brief Psychiatric Rating Scale

(BPRS); Overall & Gorham, 1962), and an increased degree of cognitive dysfunction (assessed with the Wisconsin Card Sorting Task (WCST); Grant & Berg, 1948) in 17 patients with schizophrenia.

Thus there is evidence to suggest that while the schizophrenia is associated with a hyperdopaminergic state in the striatum which may underlie the 'positive' symptoms of the disease, it is conversely associated with a hypodopaminergic state in the PFC which may underlie the 'negative' symptoms of the disease.

1.5.2 Weinberger's model of schizophrenia

In 1987, Daniel Weinberger proposed a theory that accounted for the paradoxical hyperdopaminergic state in the striatum and hypodopaminergic state in the PFC in patients with schizophrenia. Weinberger (1987) argued that a discrete region of neuropathology (in his words, a 'lesion') developed early in life in the PFC of people who went on to develop the disease. Weinberger (1987) argued that the effects of the 'lesioned' PFC remained relatively silent in childhood, that is, restricted to a number of mild behavioural abnormalities (such as delayed speech and motor development), or soft neurological signs (such as facial tics), which have been observed in children who go on to develop schizophrenia (Jones, Rodgers, Murray, & Marmot, 1994; Niemi, Suvisaari, Haukka, & Lonnqvist, 2005). Weinberger (1987) argued that this silence remained until the PFC was placed under increased functional demand in adolescence and early adulthood. Weinberger (1987) argued that this period of increased functional demand

corresponded to a period of structural brain maturation in the PFC, which interacted with the 'lesion' and caused a major disruption to the brain's dopaminergic systems. Specifically, he argued that the 'lesion' disrupted the innervation of the PFC by dopaminergic neurons in the brain stem (i.e. substantia nigra and ventral tegmental area), and that the resulting hypodopaminergic state in the PFC was responsible for the 'negative' symptoms of the disease. Furthermore, Weinberger (1987) argued that because of a feedback mechanism between the PFC and the striatum and limbic system, this reduction in PFC dopamine resulted in an excessive amount of dopamine being projected to these subcortical structures, causing hyperdopaminergia and the 'positive' symptoms of the disease. Weinberger (1987) cited work by Pycock et al. (1980), who observed that the selective de-afferentation of the PFC in rats resulted in chronically elevated levels of subcortical dopamine, as support for his theory.

1.5.3 Neurodevelopmental vs neurodegenerative theories of schizophrenia

Weinberger's (1987) theory of the pathogenesis of schizophrenia was, and remains, extremely influential in subsequent research into the aetiology of schizophrenia. There are two important points regarding Weinberger's (1987) theory that require further discussion. The first involves the proposal that damage to the brain early in life (and possibly *in utero*) is a predisposing factor to the much-later development of schizophrenia in early adulthood. This proposal is the fundamental tenet of the so-called '*neurodevelopmental*' theories of schizophrenia, of which Weinberger's was one of the first and certainly one of the most influential. Following on from the ideas of Weinberger,

a number of alternative 'neurodevelopmental' theories have subsequently been proposed in the literature. For example, in contrast to Weinberger's (1987) 'one-hit' model, which argued that a single perinatal brain insult was sufficient to precipitate the development of psychosis in early adulthood, a number of subsequent 'two-hit' models have argued that exposure to a second risk factor in the 'danger period' around adolescence and early adulthood is necessary to precipitate the onset of psychosis. Thus these 'two-hit' models subscribe to the general 'diathesis-stress' concept discussed in Section 1.4. Some 'two-hit' models have emphasised the role of certain recreational drugs (e.g. marijuana, amphetamines) as being factors potentially precipitating the onset of psychosis (Hambrecht & Hafner, 1996; Arndt, Tyrrell, Flaum, & Andreasen, 1992), while others have focused on the role of stressful life events (Cullberg, 2003; Day, 1981).

Keshavan (1999) even went so far as to propose a 'three-hit' model of the pathogenesis of schizophrenia. As with the 'two-hit' models, Keshavan (1999) argued that the presence of adverse psychosocial factors in adolescence were required for patients' pre-morbid disposition for psychosis (possibly caused by a pathological genetic profile) to become manifest. However, Keshavan (1999) went further by arguing that the neurotoxic effects associated with untreated psychosis were necessary for the development of full-blown schizophrenia, as opposed to a single psychotic episode triggered by the first two 'hits' mentioned above. The obvious implication of Keshavan's (1999) model is that it is crucial that patients with psychosis receive treatment (e.g. neuroleptics) as soon as possible after their first psychotic episode, as this may stave off the subsequent development of schizophrenia. Investigations as to the efficacy of such 'early-

intervention' treatment programs have produced equivocal results, as some studies have reported an improved patient prognosis as a result of these programs (Carbone, Harrigan, McGorry, Curry, & Elkins, 1999) while others have failed to do so (Kuipers, Holloway, Rabe-Hesketh, Tennakoon, & Croydon Outreach and Assertive Support Team (COAST), 2004).

A somewhat different 'neurodevelopmental' model was proposed by Murray et al. (1992). While Murray et al. (1992) accepted the basic 'one-hit' tenet of a perinatal brain 'lesion' interacting with the adolescent brain maturation and triggering the onset of psychosis, they argued that this model applied only to a subset of patients with schizophrenia. Murray et al. (1992) made a distinction between 'congenital' and 'late-onset' schizophrenia, and argued that while 'congenital' schizophrenia was associated with abnormal neonatal brain development, mild behavioural abnormalities and mental retardation in childhood, 'late-onset' schizophrenia was not associated with these features and was instead caused by a neurodegenerative disease process starting in middle age.

The idea that schizophrenia is associated with progressive brain atrophy over the course of the disease is the fundamental tenet of the so-called '*neurodegenerative*' theories of schizophrenia, which have become the major competitors to the 'neurodevelopmental' theories (Lieberman, 1999). The 'neurodegenerative' theories developed, at least in part, as a reaction against the somewhat fatalistic assumption implicit in the 'neurodevelopmental' theories (at least in the original 'one-hit' hypothesis) that the development of schizophrenia was inexorable given the relevant noxious perinatal

precursors, that is, that patients were "*doomed from the womb*", as coined in the phrase by Murray and Lewis (1987, p.681). It has been argued that one of the strongest pieces of evidence in favour of the 'neurodegenerative' theories of schizophrenia is the ephemeral symptom profile associated with the disease. It is well known that the symptom profile of patients with schizophrenia tends to change over the course of the disease, with the general trend being an improvement in the severity of the 'positive' symptoms and a worsening in the severity of the 'negative' symptoms (McGlashan, 1998). Illness chronicity has also been associated with a decline in patients' cognitive abilities, (Linscott, 2005; Cuesta, Peralta, & Zarzuela, 1998; Bilder et al., 1992), particularly in executive functioning (see also Appendix 1). In light of the fact that 'negative' type symptoms and deficits in executive functioning have typically been associated with frontal lobe damage in the neuropsychological literature (Lezak, 1995), it was proposed (e.g. Ashe et al., 2001) that the diminution of executive functioning skills in patients with schizophrenia and the worsening of the 'negative' symptom of the disease, arose from the progressive atrophy of the PFC. Critics of the view, however, argued that it was chronic patients' long-term exposure to neuroleptic medication that was responsible for the severity of their negative symptoms and cognitive decline rather than neurodegeneration *per se* (Hegarty, Baldessarini, Tohen, Waternaux, & Oepen, 1994). I will return to discuss the effects of the long-term exposure to neuroleptic medication in patients with chronic schizophrenia in Section 1.6.6

Proponents of the 'neurodevelopmental' hypotheses have argued that the failure to find evidence of reactive gliosis in the brains of patients with recent-onset schizophrenia

supports the idea that any brain damage associated with schizophrenia must occur well before to the onset of psychotic symptoms. The term ‘gliosis’ refers to a proliferation of astrocytes in a damaged region of the central nervous system (CNS) (Pekny & Nilsson, 2005). Astrocytes are glial cells that are involved in the repair of neural tissue and the production of protective scar tissue. Gliosis is part of the body’s natural response to unplanned neuron death, especially necrosis, which is a type of cell death typically caused by cell hypoxia, hypothermia, trauma or exposure to toxins, in which cells swell and burst their membranes (Raff, 1998). Given that neurodegenerative diseases are typically associated with gliosis (e.g. Alzheimer's disease; Liu, Erikson, & Brun, 1996), it has been suggested that the failure to find evidence of gliosis in patients with schizophrenia indicates that schizophrenia is not a neurodegenerative disease (e.g. Weinberger, 1987).

In response to this point, it has been argued that that the absence of gliosis in patients with schizophrenia could be consistent with an apoptotic neurodegenerative mechanism, as opposed to a necrotic neurodegenerative mechanism (e.g. Margolis, Chuang, & Post, 1994). Apoptosis is a form of programmed cell death in which the activation of a specific sequence of genes inside a cell causes a cascade of events that result in the cell ‘committing suicide’. Apoptosis is an essential feature of prenatal brain development in all mammals; for example, genetic mutations that inhibit apoptosis in the developing brain in mice are lethal (Kuida et al., 1996). Apoptosis is executed by ‘caspases’ present inside the cell which, when activated, cleave the nuclear membrane and the proteins that make up the cells cytoskeleton and those that attach the cell to its neighbours, and

activate the release of 'DNase' which destroys the cell's DNA (Raff, 1998; Henderson, 1996). Furthermore, cells undergoing apoptosis signal to phagocytes to come and consume their contents by flagging the cell membrane with a phospholipid molecule. Unlike necrosis, apoptosis does not result in gliosis and hence it has been suggested by a number of 'neurodegenerative' theories as being the mechanism underlying the proposed progressive brain atrophy associated with schizophrenia. Apoptosis and its possible role in the aetiology of schizophrenia will be discussed further in Chapter 4.

The 'neurodevelopmental' and 'neurodegenerative' theories of schizophrenia have often been considered mutually exclusive. However, a number of contemporary theories have attempted to combine the characteristic features of these two models. Ashe et al. (2001), for example, proposed a hybrid theory of schizophrenia that incorporated elements of both the 'neurodevelopmental' and 'neurodegenerative' models. Ashe et al. (2001) argued that genetic defects and prenatal stressors could result in the development of a disposition to schizophrenia (i.e. a diathesis). In keeping with the 'two-hit models' of schizophrenia, Ashe et al. (2001) argued that schizophrenia became manifest in adolescence when the disposed individual underwent the characteristic normative period of structural brain maturation associated with this period, combined with the associated social and environmental pressures. However, Ashe et al. (2001) diverged from the 'two-hit' models by arguing that the neuroanatomical abnormalities associated with the onset of psychosis were progressive in nature, and continued indefinitely into adulthood. Specifically, Ashe et al. (2001) proposed that a stress-triggered reduction in Brain Derived Neurotrophic Factor (BDNF), a chemical necessary for the growth and

development of neurons, was responsible for this neurodegeneration. I will discuss the role of trophic factors in the aetiology of schizophrenia further in Section 1.5.5. Similar 'hybrid' models of schizophrenia have been proposed by Murray et al. (1992) and Pantelis et al. (2003).

1.5.4 Structural brain maturation in adolescence

In Section 1.5.3, I commented that there were two important points to note about Weinberger's (1987) influential theory on the aetiology of schizophrenia, with the first being its 'neurodevelopmental' origins. The second point relates to Weinberger's emphasis of the role of periadolescent brain maturation in the onset of schizophrenia. As Weinberger (1987) commented, one of the undeniable clinical facts about schizophrenia is the *"very high probability that it will become clinically apparent in late adolescence or early adulthood"* (p.660), with the vast majority of cases occurring between the ages of 15 and 25 years for men and between 15 to 30 years for women (Jablensky, 1997).

Although it was originally thought that the development of the CNS only proceeded for the first few years after birth, subsequent studies have suggested that the periadolescent period is also a time of great structural brain change. This structural brain change is not thought to encompass a modification in the number of neurons *per se* (as neuron number is thought to remain relatively stable from early childhood until old age (Williams & Herrup, 1988)), but rather a dramatic reduction in the number of synapses. For example, Bourgeois and Rakic (1993) used an electron micrograph to count the number of

synapses in the visual cortex of macaque monkeys between 2.7 and 5 years of age (the period corresponding to their adolescence) and estimated that they were losing approximately 5000 synapses per minute in this region, over this period. Similar results have been reported in humans. For example, Huttenlocher and Dabholkar (1997) used electron microscopy to quantify the synaptic density of the middle frontal gyrus and auditory cortex in normal human brains *post mortem,* and observed an abrupt reduction in the synaptic density of the middle frontal gyrus in adolescence that was *"clearly distinct from the much later and smaller magnitude aging changes"* (p.175). Huttenlocher and Dabholkar (1997) did not find evidence of such a distinct period of synaptic elimination to occur in the auditory cortex in adolescence, which is consistent with the findings of the majority of previous studies that have reported the frontal and parietal association cortices to be most affected by this period of adolescent brain change (e.g. Sowell et al., 1999). This, of course, makes Bourgeois and Rakic's (1993) observations of enormous synaptic reductions in the primary visual cortex in adolescence even more significant, as presumably the rate of synaptic elimination would be even higher in the association cortices. The sheer magnitude of this period of brain maturation has led a number of theorists to focus on its role in the onset of psychosis and the development of schizophrenia.

1.5.5. Dysfunctional synaptic 'pruning' in schizophrenia

In a highly influential paper, Feinberg (1982) suggested that schizophrenia was caused by an abnormality in the period of periadolescent brain maturation. Citing an electron

micrograph study by Huttenlocher (1979), which indicated that there was a substantial reduction in the synaptic density in the frontal lobe associated with healthy adolescence (which was in itself a fairly radical idea at the time), Feinberg (1982) argued that this "*synaptic prune*" (p.319) arose because of a sudden decrease in the availability of the chemicals necessary for the survival of neurons and their associated synaptic infrastructure (i.e. dendrites and axon terminals). These 'chemicals' have subsequently become known as 'trophic factors' or 'neurotrophins', from the Greek word *trophé*, meaning nourishment. Brain Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF) are the two most widely investigated trophic factors in the contemporary neuroscience literature. Feinberg (1982) speculated that this 'synaptic prune' could possibly be triggered by the hormonal changes characteristic of puberty. Furthermore, he speculated that a synapse's workload, or how heavily it was used, was linked to its probability of being eliminated or preserved.

Feinberg's (1982) proposal is consistent with the work of Dale Purves, who has suggested a possible mechanism for the large-scale programmed elimination of synapses in the developing perinatal nervous system. Purves (1998) has argued that at any given synapse, stimulation by the pre-synaptic neuron causes trophic factors to be expelled into the synaptic cleft by the post-synaptic cell. These trophic factors are then taken-up by receptors on the pre-synaptic cell's axon terminals and transported retrogradely (i.e. along the axon) to the pre-synaptic cell body. Purves (1998) has argued that competition for trophic factors in the developing nervous system is the mechanism underlying synaptic elimination. Specifically, Purves (1998) has argued that the more active a

synapse is, the more likely it is to obtain a sufficient supply of trophic factor from the post-synaptic cell to ensure its survival. Purves has also suggested that the synchronicity of a synapse's activity influences its chances of survival, with synchronous activity impeding synaptic pruning and asynchronous activity enabling or even enhancing pruning (Purves & Lichtman, 1980).

In relation to the aetiology of schizophrenia, Feinberg (1982) argued that schizophrenia was caused by a dysfunction in the normative period of synaptic pruning in periadolescence, such that "*too many, too few or the wrong synapses are eliminated*" (p.331). Feinberg (1982) argued that the consequence of such a 'mis-prune' was a disintegration of neural activity, with the ubiquitous schizophrenic symptom of disorganized thinking being the most direct clinical manifestation. Following on from this idea, dysfunctions in neural integration (or in other words, abnormal neural connectivity), have formed the basis of a number of recent theories regarding the aetiology of schizophrenia. As I will discuss below, these theories have used the concept of disintegrated neural activity to explain the whole myriad of symptoms associated with schizophrenia.

1.5.6 Schizophrenia as a dysfunction in neural connectivity

Bartzokis (2002) proposed a model of schizophrenia which emphasised the role of dysfunctional neural connectivity in the development of psychotic symptoms. Bartzokis (2002) cited research which indicated that the volume of white matter in the frontal and

temporal lobe increased into the forties in healthy people (Bartzokis et al., 2001). As I will discuss further in Section 1.6.1, white matter is constituted primarily of myelin sheaths, which modulate the transmission velocity of action potentials along axons (Aboitiz, Scheibel, Fisher, & Zaidel, 1992). In light of this, Bartzokis (2002) argued that an abnormality in the processes of myelination sometime in this extensive period of brain maturation could result in a dysfunction in the normal timing of neural activity, and a disintegration in the "*formation of associations between disparate events separated in time*" (p.674) in patients with schizophrenia. Such a dysfunction in neural timing could, he argued, underlie the psychotic features of schizophrenia.

While Bartzokis (2002) refrained from speculating about the specific mechanisms by which dysfunctions in neural connectivity could give rise to symptoms of psychosis, Chris Frith has given this issue extensive consideration. Frith (1992) argued that a number of the 'positive' symptoms of schizophrenia arose from a deficit in 'self-monitoring', or an inability to recognize one's self-generated thoughts and actions as being self-generated. For example, Frith (1992) considered the very common schizophrenic delusion of 'alien control', in which patients believe that they are not in control of their body, but that some external force is compelling them to move – like a puppet being manipulated by a puppeteer. For example: *"The force moved my lips. I began to speak. The words were made for me"* (Frith, 1992, p.66). Frith (1992) argued that delusions of 'alien control', arose when patients experienced their body moving before they became aware of their intention to move it. Frith (1992) speculated that a dysfunction in the neural connectivity between the frontal and parietal lobes was the

physiological underpinning of delusions of ‘alien control’. Frith, Blakemore and Wolpert (2000) cited evidence suggesting that while externally generated actions (e.g. someone else picking up your hand and moving it) were associated with high activity in the somatosensory and neighbouring superior parietal cortex, internally generated actions were associated with low activity in these regions (Spence et al., 1997). Frith, Blakemore and Wolpert (2000) argued that this was because self-generated actions initiated in the supplementary motor area (SMA) (Passingham, 1993) caused the inhibition of activity in parietal cortex by means of so-called ‘corollary discharges’ from the SMA neurons. They cited a supporting study that showed that when healthy subjects tickled themselves it resulted in minimal activation of the aforementioned parietal areas compared to when these subjects were tickled by someone else (Blakemore, Wolpert, & Frith, 1998). Frith, Blakemore and Wolpert (2000) argued that abnormalities in these ‘corollary discharges’ were responsible for delusions of ‘alien control’, as the failure to inhibit parietal activity led to self-generated movements being perceived as being externally generated. An alternative explanation of this phenomenon is that there is an abnormality in the *timing* of the corollary discharges, such that the parietal cortex activity is inhibited by the SMA neurons after the bodily movement has actually occurred. This could be imagined as your hand moving before you become aware of your intention to move it. Such an abnormality in neural timing could result from a dysfunction in the processes of the myelination of these frontal-parietal projections, in line with the theory proposed by Bartzokis (2002).

It may initially seem as though the explanatory power of a theory focusing on self-monitoring deficits in patients with schizophrenia is limited to explaining delusions of

'alien control'. Frith (1992), however, has argued that a similar mechanism underlies a number of the other 'positive' symptoms of schizophrenia. For example, he proposed that a failure to recognize the self-generated nature of one's own thoughts could lead to symptoms of thought insertion, in which patients experience thoughts coming into their mind from an outside source, or in auditory hallucinations, in which self-generated sub-vocalisations are perceived as being externally generated voices.

Nancy Andreasen also proposed a model of schizophrenia that emphasized the role of abnormal neural connectivity. Consistent with the ideas of Bleuler (1911), Andreasen (1999) argued that the fundamental abnormality in schizophrenia was a loosening of associations between cognitions or, as she put it, a 'cognitive dysmetria' which was the "*disruption of the fluid, coordinated sequences of thought and action that are the hallmark of normal cognition*" (p.784). Specifically, Andreasen (1999) argued that this 'cognitive dysmetria' arose because of a breakdown in neural connectivity between the cerebral cortex (and particularly the PFC) and the cerebellum, a loop that was mediated by the thalamus. In saying this, Andreasen (1999) was one of the first theorists to argue that the cerebellum did more than coordinate motor activity, as had long been thought to be its sole responsibility. Andreasen (1999) argued that this 'cognitive dysmetria' underlay all of the symptoms of schizophrenia. She argued that the breakdown in connectivity between the cortex and the cerebellum in schizophrenia led to patients:

1) making inappropriate associations between mental representations – which resulted in disorganized thought and behaviour, persecutory delusions and inappropriate affect,
2) losing the ability to distinguish between their self and the external world – which resulted in auditory hallucination and certain delusions as per Frith (1992), and,
3) losing the ability to distinguish between important and trivial stimuli – which resulted in delusions of reference, poor communication skills etcetera.

Furthermore, Andreasen (1999) argued that difficulties in inhibiting the relevant from the irrelevant could also result in some of the 'negative symptoms' of schizophrenia, such as avolition and alogia, as it could cause patients to 'freeze up', "*much as a computer locks up when it cannot match signals sent at an incorrect rate or to an incorrect place*" (p.785).

One final, and controversial, theory of schizophrenia proposed by Tim Crow emphasized the role of aberrant functional and structural brain *laterality* in the aetiology of the disease, specifically in those brain regions specialized for language. Crow (1997) considered the fact that while schizophrenia undoubtedly has a strong genetic component (with previous studies indicating that the monozygotic twins of schizophrenic patients have a greater than 40% chance of developing the disease themselves – a rate 40 times higher than the general population (Gottesman, McGuffin, & Farmer, 1987)), it is also clear that patients with schizophrenia have far fewer children on average than do the rest of the population (McGrath et al., 1999). Crow (1997) began his theory by asking why it

was that the genes responsible for schizophrenia had not been 'bred out' over time, and why the rates of schizophrenia were generally reported as being consistent and stable over time and across cultures (Jablensky, 1997). Crow (1997) concluded that the genetic variation that gave rise to schizophrenia must be concordantly associated with a behavioural feature characteristic of the human species, and he proposed that this feature was the ability to use language. As Berlim et al. (2003) commented "*psychosis and language are both linked to the genetic change that originated the species*" (p.9).

Crow (1997) argued that the development of language was enabled by the development of structural asymmetries in the hemispheres of the human brain. Crow (1997) cited the fact that the vast majority of the general population (and an even greater proportion of right handed people) exhibited a left-greater-than-right pattern when it came to the volume of the posterior temporal lobe and anterior parietal lobe structures, (e.g. the superior temporal gyrus, planum temporale, Wernike's area), which have long been known to be involved in the processing and comprehension of language (Harasty, Double, Halliday, Kril, & McRitchie, 1997; Naeser, Helm-Estabrooks, Haas, Auerbach, & Srinivasan, 1987). Crow (1997) argued that this structural asymmetry was indicative of an adaptive *functional* asymmetry, by which time-critical aspects of language were processed in the same hemisphere in order to maximise processing speed (Ringo, Doty, Demeter, & Simard, 1994). Citing a number of studies that indicated that patients with schizophrenia exhibited less structural brain asymmetry in the language centres than did healthy people, Crow (1997) argued that patients with schizophrenia exhibited a failure of hemispheric dominance for language. Put simply, Crow (1997) argued that in patients

with schizophrenia, the time delay inherent in having to use both hemispheres to process language led to abnormalities in neural timing, and subsequently the symptoms of schizophrenia. In this sense, the concept of dysfunctional neural connectivity also lay at the heart of Crow's (1997) model, although the mechanism he proposed for this dysfunctional connectivity was very different from those outlined in connectivity models proposed by Bartzokis (2002), Frith (1992) and Andreasen (1999).

1.6 THE NEUROANATOMICAL UNDERPINNINGS OF SCHIZOPHRENIA

From the above discussion it may appear as though the major contemporary biological theories of schizophrenia have very little in common with each other. For example, while Weinberger (1982) emphasized the significance of a perinatal 'lesion' in the PFC in the development of schizophrenia, Feinberg (1982) emphasized the role of dysfunctional synaptic 'pruning' in adolescence. Bartzokis (2002), on the other hand, focused on abnormalities in axonal myelination in patients with schizophrenia, while Crow (1997) emphasized the significance of abnormalities in the lateralisation of the brain's language centres, and so on. In spite of the significant differences between them, however, all of these theories have one feature in common – **they all explicitly propose the existence of specific neuroanatomical abnormalities in patients with schizophrenia**. The primary aim of this thesis was to identify the specific structural brain abnormalities exhibited by patients with schizophrenia. The following section aims to describe the methods that have

been used in the literature to look for evidence of structural brain abnormalities in patients with schizophrenia.

1.6.1 Identifying and quantifying neuroanatomical abnormalities in patients with schizophrenia

Given the long history of theorizing as to the origins of schizophrenia, it is perhaps not surprising that there has been a correspondingly long history of research examining the structural brain abnormalities associated with the disease. The first investigations of the neuropathology of schizophrenia were *post mortem* studies that took place in the late 19th and early 20th centuries (Shenton, Dickey, Frumin, & McCarley, 2001). The methodology employed by most of these early studies involved removing the deceased patient's brain, dissecting it and comparing it qualitatively to the brains of deceased 'normal' individuals. Despite their crude methodologies, even these early studies were able to distinguish between three primary types of brain tissue. The first, which was dubbed *grey matter* because of its greyish-brown appearance, was concentrated in the middle of the brain and also constituted its outermost layer (which was dubbed the 'cortex' from the Latin word for 'bark'). Subsequent research identified the grey matter as being constituted mostly of neuron bodies and their associated dendrites and axon terminals (Carlson, 2002). The second tissue type, dubbed *white matter* because of its whitish appearance was observed to lie primarily between the cortical and subcortical grey matter. The fact that white matter was observed to be continuous with the spinal cord, which was known to relay sensory information from the body to the brain, led to these early researchers making the

correct assumption that the white matter relayed information to and from the cell bodies in the grey matter (Bear, Connors, & Paradiso, 1996). Subsequent research identified that white matter as being constituted primarily of the myelinated sheaths of the axons corresponding to the neuron bodies in the grey matter. The third 'tissue' type, dubbed *cerebrospinal fluid (CSF)*, was observed to be a clear fluid that surrounded the brain and filled its inner cavities or 'ventricles'. Subsequent research identified the primary functions of the CSF as being a 'shock-absorber' for the brain and also enabling the transport of nutrients to the brain and the transport of metabolites away from it (Kandel, Schwartz, & Jessell, 2000). A *post mortem* slice of the human brain in which the grey matter, white matter and ventricles (which are filled with CSF in the living brain) are clearly distinguishable is illustrated in Figure 1-1.

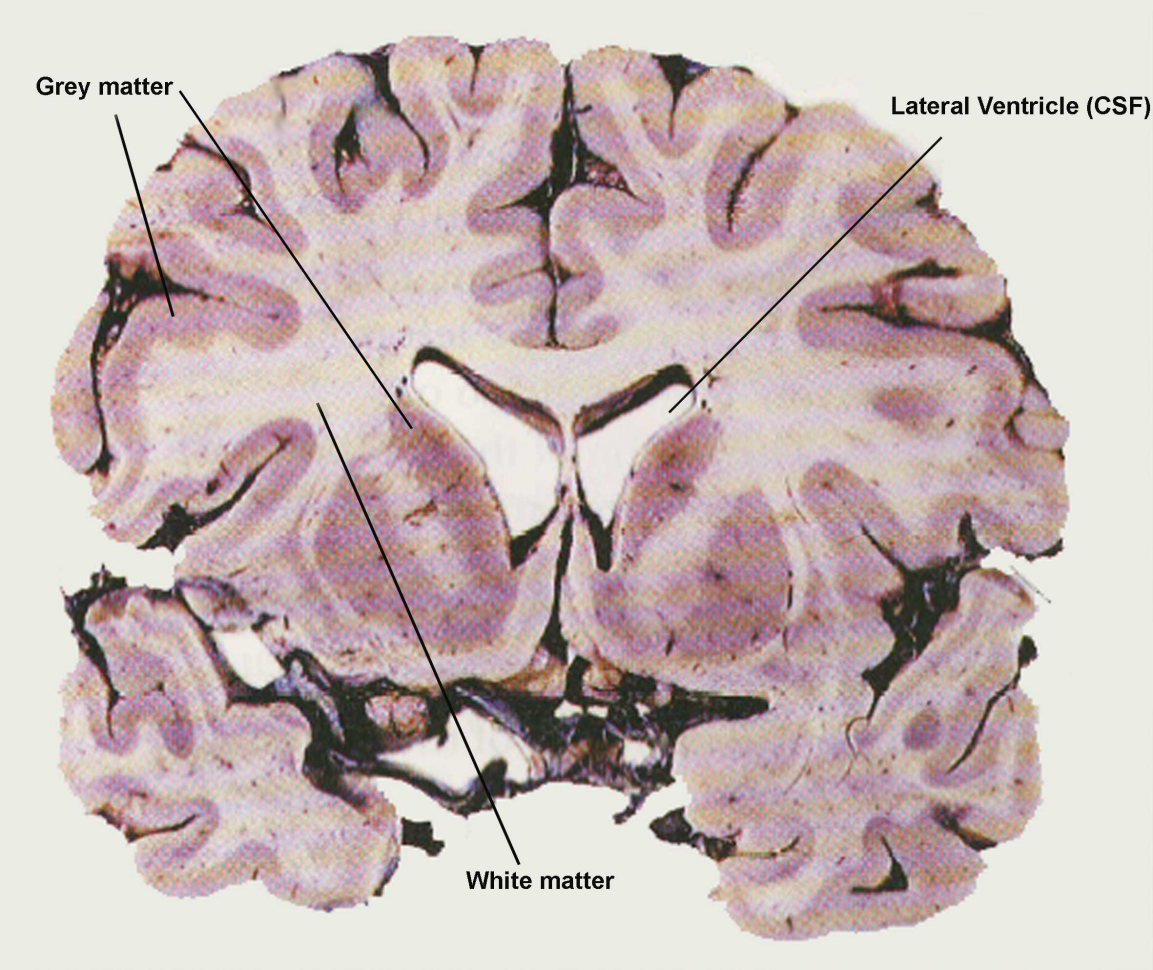

Figure 1-1: A *post mortem* slice of the human brain with the grey matter, white matter and ventricles (which are filled with cerebrospinal fluid in the living brain) clearly delineated. Image from Carlson et al. (2002)

The majority of these early *post mortem* studies reported little evidence of neuroanatomical abnormalities in patients with schizophrenia, at least in comparison to the substantial pathologies that were observed in patients suffering from some of the other neurological disorders being studied at the time, such as Alzheimer's disease (Dunlap, 1924). And while some studies reported a slight atrophy of the cerebral cortex, particularly in the frontal lobe (e.g. Southard, 1914), and an enlargement of the lateral ventricles (Jacobi and Winkler 1928, as cited in Shenton et al., 2001) in patients with

schizophrenia, the majority did not and the dearth of supporting studies led to the widely held belief that "*There is no neuropathology of schizophrenia*" (title of the first International Congress of Neuropathology, Rome, 1952, as cited in Bogerts, 1999), and that "*schizophrenia is the graveyard of neuropathologists*" (Plum, 1972).

This perception changed, however, with the advent of Computer Assisted Tomography (CT) in 1973 (Hounsfield, 1973). Computer Assisted Tomography was the first of the so-called 'neuroimaging' techniques, which enabled the visualization of brain structure in a living person. The CT scanner worked by passing a beam of X-ray radiation through the patient's brain and measuring with a detector the energy of the X-rays that emerged out the other side. Dense tissues such as bone tended to absorb more of the X-rays' energy (and hence allowed less energy through to the detector) than less dense tissues such as CSF. The X-ray generator (and the corresponding detector) moved 360º around the patient's head and thus enabled the construction of a 2-dimensional cross-section of the brain. By acquiring a number of cross-sectional images through the patient's brain, a 3-dimensional CT image could be generated in which differences in image intensity enabled the differentiation of the brain's constituent tissue types. A CT image of a living human brain is provided in Figure 1-2.

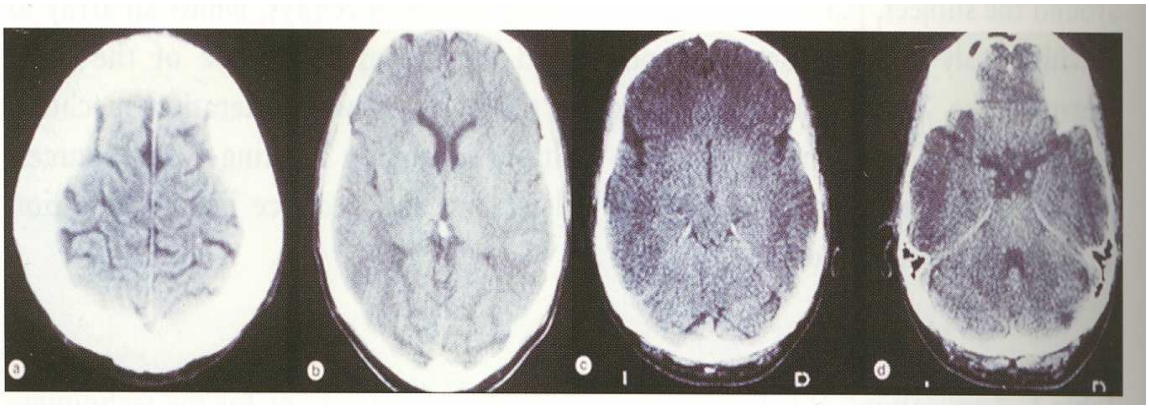

Figure 1-2: A CT scan of the living human brain. Whilst the skull and ventricles can be clearly distinguished from the underlying brain tissue, it is more difficult to distinguish between the grey and white matter of the brain. Image from Sharma and Chitnis (2000)

The first CT investigation of structural brain abnormality in patients with schizophrenia was performed by Johnstone et al. (1976), who reported enlarged lateral ventricles in the patient group compared to matched controls. This study initiated a flurry of CT research, with enlarged ventricles, enlarged frontal lobe sulci, and temporal lobe atrophy being commonly reported (see reviews by Raz, 1993; Shelton & Weinberger, 1986). Whilst studies employing CT had a major advantage over *post mortem* studies in that they avoided the confounds associated with investigating a dead brain (such as the shrinkage and swelling of brain tissue, and brain changes secondary to neurological or vascular diseases (Falkai & Bogerts, 1995)), their major limitation lay in the fact that while CT provided a good contrast between skull, brain tissue and CSF, it did not provide a good contrast between grey and white matter. This made the distinction between the subcortical grey matter structures and the neighbouring white matter especially difficult (Sharma & Chitnis, 2000).

This limitation was overcome in the mid-1980s with the development of a new neuroimaging technique known as Magnetic Resonance Imaging (MRI). Magnetic Resonance images provided a much clearer delineation between the grey and white matter of the brain than did CT images. In recent years, MRI has become the most widely used neuroimaging technique for structural brain research, although CT is still widely used clinically thanks largely to its being considerably less expensive than MRI. In this thesis I used the neuroimaging technique of MRI to identify and quantify the structural brain abnormalities present in patients with schizophrenia. In the following section I will briefly review the principles of MRI, and describe the ways it can be used to identify and quantify structural brain abnormalities.

1.6.2 Principles of Magnetic Resonance Imaging (MRI)

The MRI scanner is built around a powerful magnet, with a typical magnetic field strength of one to three Tesla. Magnetic Resonance Imaging works, in essence, because of the fact that the three constituent tissues of the brain (i.e. grey matter, white matter and CSF) have different magnetic properties.

Protons have an inherent property called ‘spin’, which is often described as being analogous to the spinning of a child’s top (Friedmann, Jones, Chavez-Munoz, Salmon, & Merritt, 1989). As protons are charged particles, their spin generates a tiny magnetic field, which leads to protons being dipolar, that is, they have a north and a south pole. The magnetic orientations of protons in the brain are normally randomly distributed,

however in the presence of an external magnetic field (such as that produced by an MRI machine) the protons will align themselves either parallel or anti-parallel with the direction of the magnetic field. Due to its being a lower energy state, a slightly higher proportion of protons align themselves parallel to the magnetic field, and thus there is a resultant slight net magnetization parallel to the axis of the magnet. Specifically, it has been estimated that for every 10 000 000 protons aligned anti-parallel to the magnetic field, 10 000 007 are aligned parallel (Schild, 1990). The resultant net magnetization parallel to the magnetic field is referred to as the *longitudinal magnetization*.

The external magnetic field generated by the scanner also causes the protons to 'spin' differently than they would normally; rather than spinning stably on their axis, the external magnetic field causes the protons to 'wobble', like a child's top that has been bumped (Schild, 1990). This characteristic 'wobbling' movement of protons is referred to as 'precession'. The speed at which protons precess (i.e. the precession frequency) is related to the strength of the external magnetic field, with higher magnetic field strengths causing higher precession frequencies. While all protons will precess at the same frequency given a constant magnetic field strength, they will not all be the same points in their orbit round their axis, that is, they will not all be precessing in phase.

The administration of a pulse of electromagnetic radiation in the radio frequency (RF) band at the exact precession frequency of the protons causes two things to occur. Firstly, it causes some of the protons aligned parallel to the magnetic field to absorb energy and flip to being anti-parallel. This reduces the size of the longitudinal magnetization.

Secondly, it causes the protons to begin to precess in phase. This produces a so-called *transverse magnetization* that spirals with the precession of the protons. When the RF pulse is turned off, and the behaviour of the protons is once again determined by the external magnetic field, two processes simultaneously occur. Firstly, protons that were flipped to being anti-parallel to the external magnetic field return to being parallel, and thus the longitudinal magnetization increases to its value prior to the RF pulse. This process is known as *T1-relaxation*. Secondly, the protons stop precessing in phase and start to de-phase. This results in a loss in transverse magnetization to its value prior to the RF pulse (i.e. to zero), and this process is known as *T2-relaxation*. It should be emphasized that these two processes occur simultaneously and independently after the RF pulse is turned off. The changing magnetization associated with the T1 and T2-relaxation induces an electrical current that is detected by an antenna (known as the *receiver coil*) that is placed around the patient's head. This electrical current (or MR signal) is the signal used in MRI. By manipulating the parameters of the scanning sequence such as repetition time (TR – the time between sequential RF pulses) and the echo time (TE – the time between the first (of at least two) RF pulses and the sampling of the MR signal), it is possible to bias the scan towards emphasizing differences in longitudinal or transverse relaxation. Thus a T1-weighted scan emphasizes differences in T1-relaxation while a T2-weighted scan emphasizes differences in T2-relaxation.

The scanning sequence that I have used in all of the studies in this thesis is a typical T1-weighted scan that has been dubbed the 'MPRAGE' scan (for a general overview of the MPRAGE sequence see http://www.mri.jhmi.edu/~craig/protocols/mprage.html). It is

possible to employ a T1-weighted scan when imaging the brain because of the fact that the three constituent tissue types (i.e. grey matter, white matter and CSF) each show differences in T1-relaxation. Of the three tissue types, white matter has the fastest T1-relaxation time (i.e. the shortest time for the longitudinal magnetization to return to its pre-RF levels) due to its high concentration of protons in the fatty myelin sheath surrounding the axons. Cerebrospinal fluid, on the other hand, has the slowest T1-relaxation time of the three tissue types, due to the low proton density inherent in its high water content. Grey matter has a T1-relaxation time between that of white matter and CSF. Thus when sampling the MR signal at a given time after the RF pulse, WM will have a higher longitudinal magnetization than grey matter that will have a higher longitudinal magnetization than CSF. In light of the fact that a high longitudinal magnetization is associated with an intense MR signal, white matter appears bright or white on an MR image, while CSF appears dark or black. Grey matter appears as an intermediate intensity between white and black, i.e. grey. A typical MPRAGE scan of the human brain is presented in Figure 1-3. As can be seen, the MR image is of a far superior resolution to the CT image shown in Figure 1-2, and shows a clear demarcation between the white matter, grey matter and CSF.

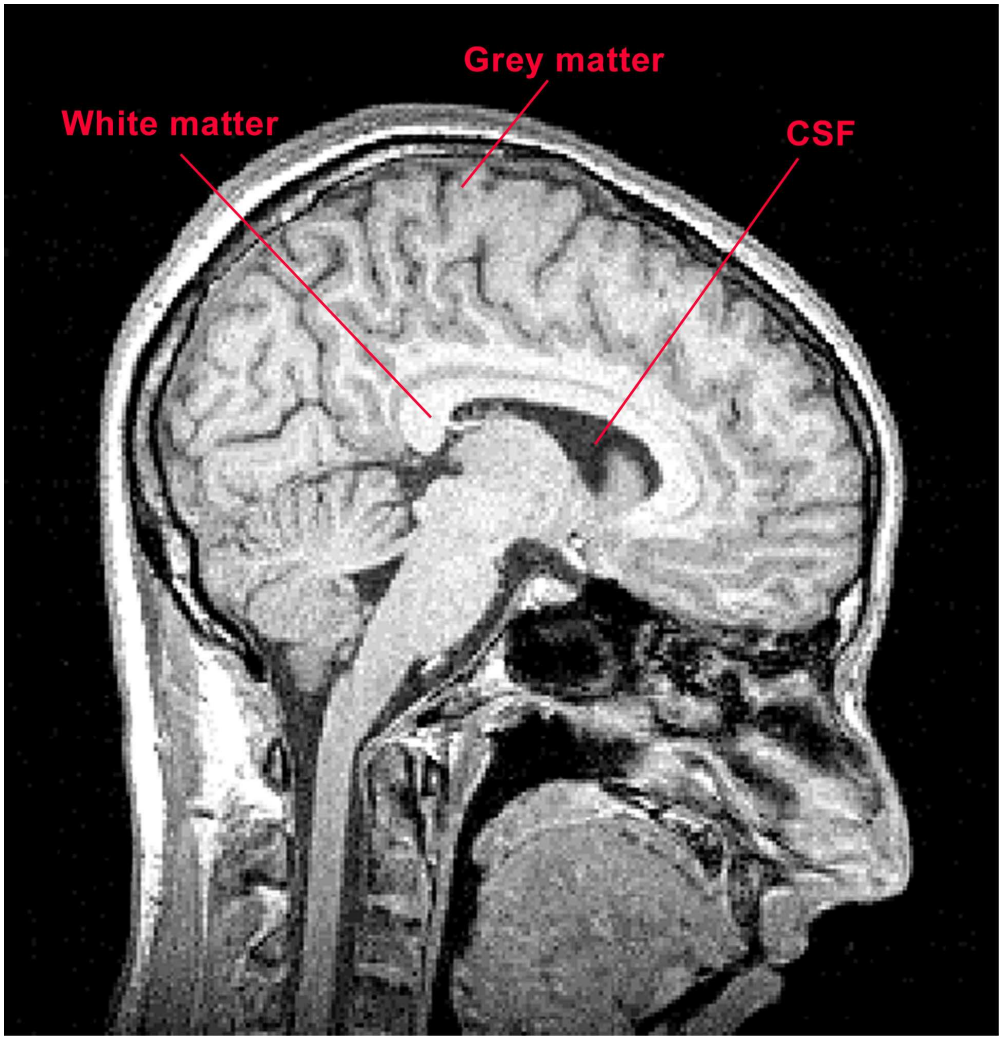

Figure 1-3: A T1-weighted MR image of the living human brain (the brain of the author, in fact!). MRI allows for a clear delineation between the three fundamental tissue types of the brain: the grey matter (e.g. in the parietal cortex), white matter (e.g. in the corpus callosum), and cerebrospinal fluid (e.g. in the lateral ventricles)

Since the development of MRI in the mid-1980s it has become the most popular technique for assessing and most importantly *quantifying* evidence of structural brain abnormalities in all manner of psychological and neurological conditions including Alzheimer's disease (Lee, Mintun, Buckner, & Morris, 2003), Huntington's disease (Kassubek et al., 2004), Parkinson's disease (Brooks, 2000), depression (Kanner, 2004), obsessive-compulsive disorder (Valente, Jr. et al., 2005), bipolar disorder (Monkul,

Malhi, & Soares, 2005) and, of course, schizophrenia (Kasai et al., 2002; McCarley et al., 1999; Pearlson & Marsh, 1999). There are two general techniques that have been widely used to assess and quantify evidence of structural brain abnormalities in patients with schizophrenia on the basis of MR images. These techniques have been referred to as the 'Region-of-Interest' (ROI) approach and the 'statistical imaging' (SI) approach.

1.6.3 Assessing neuroanatomical abnormalities on the basis of MR images: the Region-of-Interest (ROI) approach

Assessing the existence and extent of neuroanatomical abnormalities in patients with schizophrenia using the Region-of-Interest (ROI) approach is conceptually very simple. The technique involves taking a 3-dimensional MRI scan and manually tracing the structure or region of interest on each individual slice. For example, the region being traced in Figure 1-4 (from a study by Lee et al., 2002) is the fusiform gyrus.

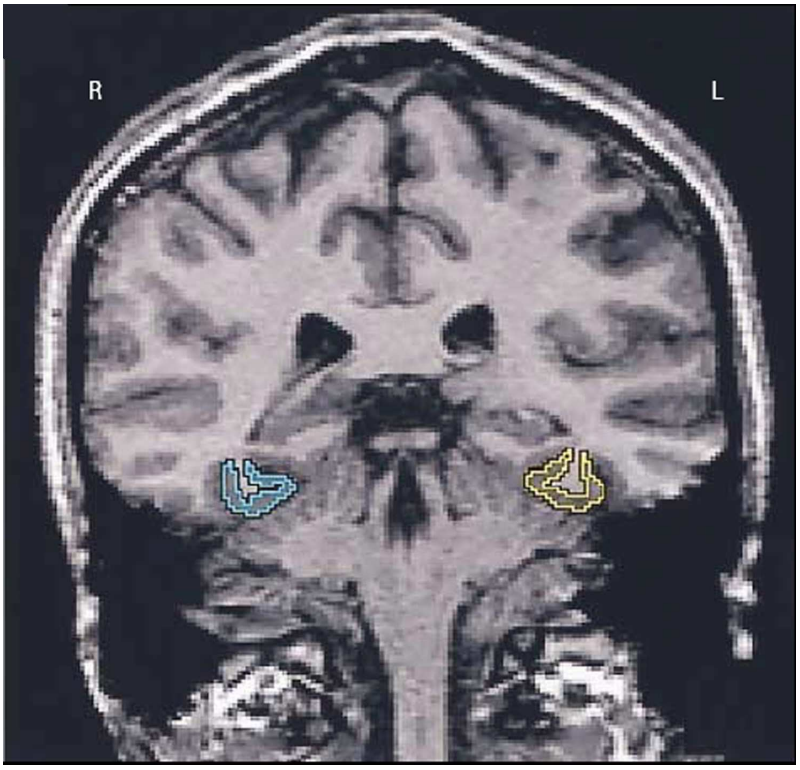

Figure 1-4: Using the Region-of-Interest (ROI) approach to investigate the neuroanatomical underpinnings of schizophrenia. In this example, the fusiform gyrus is being defined as the ROI. Image from Lee et al. (2002)

By calculating the area of the traced ROI in each slice (which is normally done by counting the number of constituent pixels, with the help of a MRI analysis software package such as MRICRO (Chris Rorden, http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html)), and multiplying this by the slice thickness (which is a parameter of the MRI acquisition), it is possible to calculate the volume of a given structure/region for each participant, and thus compare the volume of this structure/region between a patient and control group.

The major advantages of using an ROI approach is that it is a simple technique to implement, and does not require the warping of images, such as is required by the

'statistical imaging' techniques described below. There are, however, a number of disadvantages of the ROI approach. The first disadvantage is that it is a very time-intensive process. Given a slice thickness of 1 mm (a reasonably standard resolution for T1-weighted MRI scans), a subject's brain can be covered with between approximately 180 to 250 slices, depending on the plane that the slices are acquired in. As the ROI approach requires that a structure be traced on every individual brain slice that it can be observed on (or every third or fourth slice if the structure is very similar from slice-to-slice), then it is clear that if the structure extends over a large proportion of the total number of slices (e.g. the fusiform gyrus), then the amount of time required to trace this structure for even a single subject (let alone for the 30 or more subjects required per group in order to ensure a reasonable sample size) would be considerable. A second disadvantage of the ROI approach is that an ROI study is necessarily limited to the regions defined by prior hypotheses. This can be a disadvantage if the regions affected by a particular disease are widespread or unknown, such as in schizophrenia. As it is quite feasible (indeed highly likely, as will be discussed in Sections 1.7.1 and 1.7.2), that rather than being associated with structural brain abnormalities limited to a few discrete ROIs, schizophrenia is instead associated with widespread structural abnormalities, then the ROI approach is limited in its capacity to describe 'the whole story' regarding the structural brain pathologies associated with the disease. Furthermore, it is also feasible that the structural abnormalities present in patients with schizophrenia may not be restricted to, or conform to, the contrast-delineated boundaries that are typically used to differentiate between ROIs.

1.6.4 Assessing neuroanatomical abnormalities on the basis of MR images: the 'Statistical Imaging' (SI) approach

For identifying and quantifying structural brain abnormalities on the basis of MR images, the most widely used alternative to the ROI approach is the 'statistical imaging' (SI) approach. The defining feature of the SI techniques is their division of the MR image into hundreds of thousands of *voxels* (literally a 'volume pixel', or a 3-dimensional pixel), followed by the performance of a statistical analysis *at each voxel* in the image. Thus the SI techniques (given appropriate statistical correction for multiple comparisons) are able to investigate for evidence of structural brain abnormalities at every voxel in an MR image, and thus overcome the second limitation of the ROI techniques described above. Other advantages of the SI techniques are that 1) they are fast to implement which allows for larger sample sizes, and 2) they are automatically implemented and do not require the manual tracing of ROIs by a researcher, which means that they are, in this sense, unbiased. Proponents of the SI techniques generally argue that they provide a higher degree of consistency between subjects and between studies than the ROI techniques, however this point is debatable and has been contested by a number of researchers in the literature (Giuliani, Calhoun, Pearlson, Francis, & Buchanan, 2005).

The major disadvantage of the SI techniques is that they require all the brain images in a study to have the same global shape. That is, they require all images to correspond to a uniform coordinate system. For example, the SI techniques require that the voxel at a coordinate [x,y,z], located in the amygdala (for example) in subject 1 to also be located at

[x,y,z] and to also lie in the amygdala in subject 2, and in subject 3 and subject 4 and so on. As there is a considerable amount of variation that naturally exists between individual brains, the fulfilment of this requirement requires the individual images to be warped to the same global shape, which is usually defined by some template image. In order for the SI techniques to produce accurate results, this warping procedure (which has been referred to in the literature as *spatial normalization*) must eliminate differences in global brain shape while preserving local differences in tissue constituency. A number of spatial normalization algorithms have been designed that aim to achieve this goal, and the question of which algorithm is the best (or indeed whether the spatial normalization procedure results in valid data at all) remains a matter of hot debate in the neuroimaging community (see, for example, the debate between Ashburner and Friston (2001) and Bookstein (2001)). In spite of the ongoing debate, however, the SI techniques have become very widely used in the neuroimaging community over the past decade. The SI technique that is most commonly used worldwide for research into the neuroanatomical underpinnings of schizophrenia is known as 'voxel-based morphometry' (VBM), and the Statistical Parametric Mapping (SPM) software package (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm) is by far the most popular software package for implementing this technique. In this thesis I used the SPM software package to implement the technique of VBM for all my experimental studies. The following section will provide a brief overview of the procedures involved in performing VBM with SPM.

1.6.5 Voxel-based morphometry with SPM

The Statistical Parametric Mapping (SPM) software package was originally developed by Karl Friston and colleagues in 1991 (see Friston, Frith, Liddle, & Frackowiak, 1991). Subsequent to the release of the original version (SPMclassic), a number of updated versions have been released to the public (SPM94, SPM95, SPM96, SPM99, SPM2 and, recently, SPM5). Although it was initially developed for the analysis of functional imaging data (i.e. PET scans), the SPM software package also enables the automatic implementation of voxel-based morphometry (VBM). There are a number of procedures involved in the implementation of voxel-based morphometry using SPM, namely spatial normalization, segmentation, cleaning, Jacobian modulation, smoothing and statistical analysis. Each of these procedures is briefly described in the following section. Figure 1-5 provides a diagrammatic summary of the procedures involved in VBM using SPM.

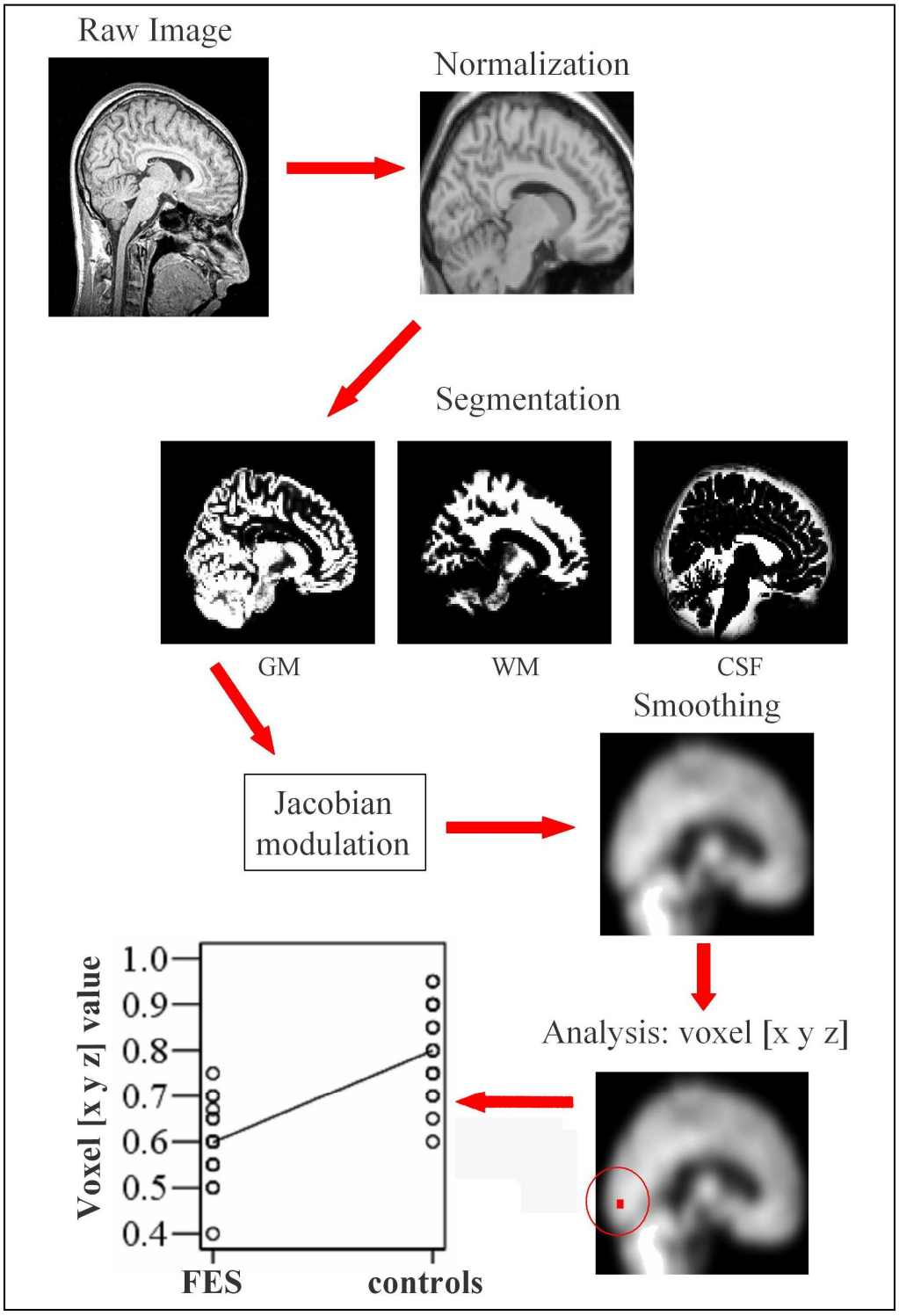

Figure 1-5: A diagrammatical summary of the analytic technique of voxel-based morphometry (VBM), which was implemented with the Statistical Parametric Mapping (SPM) software package in this thesis. The arrows track the normalization of the raw images to a template image, the segmentation of the normalized images and the subsequent Jacobian modulation, smoothing and voxel-wise analysis of the grey matter segment

1.6.5.1 Spatial Normalization: The procedure of spatial normalization involves the warping of the MR images of all subjects in a given study into the same global space. This is achieved by warping the images of all subjects to that of an 'average brain' or template image.

Talairach and Tournoux (Talairach & Tournoux, 1988) were among the first to provide a standardized coordinate system for the brain, which they published in the form of a brain-atlas. Subsequently, a number of SI studies have used the "Talairach brain" as their normalization template (e.g. see Chau & McIntosh, 2005). The main advantage of performing MRI analysis in a standard stereotactic space is that it allows a direct comparison of experimental results from different laboratories in the same coordinate system. However, there are limitations to using the Talairach brain as a normalization template. The main criticism of the Talairach atlas is that it was created based on the post-mortem brain of a single, 60 year old French woman, and thus does not provide a good representation of the neuroanatomy of the general population. As a result of this, many research groups have chosen to normalise their MR images to a more representative template image. One of the more popular templates (and the template used in SPM) is known as known as the Montreal Neurological Institute T1-weighted template (or just the MNI template; McGill University, Quebec, Canada, http://www.mni.mcgill.ca/). The MNI template was averaged from the T1-weighted images of 152 healthy subjects, of which 57% were male, 90% were right handed and with a mean age of 25 years. In spite of this, the Talairach coordinate system has remained the standard reference for reporting brain locations in the neuroimaging

literature (Chau et al., 2005), notwithstanding the fact that a number of atlases are now available which outline the coordinate system of the MNI brain (Hammers et al., 2003). Thus, in order to enable consistency between studies, it has become increasingly popular for researchers to normalize their brain to an averaged template brain (e.g. the MNI brain) but then transform and report their findings in the Talairach coordinate system. An algorithm written by Matthew Brett and colleagues has been designed specifically to transform coordinates from MNI to Talairach space (http://www.mrc-cbu.cam.ac.uk/Imaging/Common), and has been employed extensively throughout this thesis.

As the MNI template is averaged from the images of 'normal' brains, it should be noted that it is not always preferable to normalize to this template directly, particularly when investigating the differences between 'normal' (i.e. control) and 'abnormal' (i.e. patient) brains, because of the inherent systematic bias (i.e. the patient images will tend to undergo more warping than the control images). In this case, it is preferable to generate a study-specific template averaged from the patient and control images used in the particular study, which have each previously been warped to the MNI template. Warping to images that have already been warped to the MNI template is useful, as while there are a number of atlases available that outline the coordinate system of the MNI brain (e.g. see Hammers et al., 2003), there is obviously no atlas available that describes the coordinate system of a study-specific template.

The procedure of spatial normalization involves minimizing the residual sum of squared differences between the template image and each subject's MR image (Ashburner & Friston, 2000). There are two steps involved in spatial normalization. The first step involves a linear registration, which aims to account for linear shape differences between the subjects' image and the template image. The linear registration involves estimating the optimum 12 parameter linear (affine) transformation required to match each subjects' image to the template. The 12 parameters consist of a rigid-body transformation (i.e. 3 translations (movement of voxels in the x, y and z planes) and 3 rotations (rotating voxels in the three planes)), as well as 3 zooms (enlarging/contracting voxels in the three planes) and 3 shears (when the opposing faces of the voxels are moved in opposing directions in the three planes).

The second step involved in spatial normalisation is a non-linear registration, which aims to account for non-linear, global shape differences between the subjects' image and the template image. Non-linear registration is implemented via the use of a number of low-frequency (i.e. global) deformations, via a linear combination of smooth spatial basis functions (Ashburner & Friston, 1999). Local differences in brain morphology are not accounted for by this non-linear registration. This imperfect registration of subject images to the template is a necessary condition for the implementation of VBM, as a perfect registration would eliminate all differences between the subjects and the template image, down to as fine a scale as voxel-level intensity values. Given that the statistical analysis in SPM is based on differences in voxel intensity values, this is obviously undesirable, as

it would mean that all subjects' images would appear identical and any between-group differences would be artificially eliminated.

Following spatial normalization, all subjects' images must be re-sliced into isotropic voxels, as any voxels warped during the registration procedures may not have remained cubic. The important point to realise is that subsequent to normalization and reslicing, all subjects' images have the same global shape and have the same number of voxels, and that the only differences that are preserved between the images are local differences in voxel intensity.

1.6.5.2 Segmentation: Segmentation in SPM involves the partitioning of a spatially normalized MR image into its three constituent tissue classes: grey matter, white matter and cerebrospinal fluid (CSF). The three constituent tissue types of the brain can be distinguished on a T1-weighted MR image by their signal intensities: CSF has a low intensity value and appears black on a T1-weighted image, white matter has a high intensity value and appears white on a T1-weighted image, while grey matter has an intensity value between that of white matter and CSF, and thus appears grey. SPM employs a 'mixture-model' cluster analysis technique for its segmentation procedure, which means that in addition to segmenting the MR image on the basis of differences in signal intensity, SPM also segments on the basis of an *a priori* expectation of what tissue type it expects to find at each voxel in the brain. This approximate knowledge of the spatial distributions of the three tissue types in the brain comes from probability images generated by the Montreal Neurological Institute, that have been derived from the

averaged MR images of over 300 ‘normal’ brains (Evans, Kambler, Collins, & Macdonald, 1994). The end products of this segmentation procedure are three ‘probability maps’ – one for grey matter, one for white matter and one for CSF. In the grey matter probability map, for example, the value at a given voxel represents the probability of that voxel being grey matter. That is, after segmentation, each voxel has ‘probability’ value between 0 and 1 where a value of 0 would indicate that the voxel was definitely not grey matter while a value of 1 would indicate that the voxel definitely was grey matter. Voxel values are typically observed to be either close to 0 (i.e. very unlikely to be grey matter) or very close to 1 (i.e. very likely to be grey matter). The same applies for the white matter and CSF probability maps.

1.6.5.3 Cleaning (removal of extra-cerebral tissue): The segmentation procedure can sometimes incorrectly classify some voxels of extra-cerebral tissue (e.g. skull, scalp, meninges, venous sinuses) as being voxels of grey matter, white matter or CSF. The ‘cleaning’ procedure in SPM aims at manually removing these extra-cerebral voxels from the segmented probability images discussed above. The extra-cerebral voxels are removed from the segmented images with the help of a binary image of the cerebrum, in which all cerebral voxels are designated a value of 1 and all extra-cerebral voxels are designated a value of 0. The binary image of the cerebrum (which is individually created for each subject) is generated by initially eroding the white matter image in order to remove any non-contiguous voxels. This cleaned white matter image is then dilated until it reaches the cortical CSF that marks the outermost extent of the cortical grey matter, creating a binary image of the cerebrum. Extra-cerebral voxels are removed for each

subject by convolving their grey and white matter images with their binary image of the cerebrum. Following the removal of the extra-cerebral tissue, the probability of any given voxel being grey matter plus the probability of it being white matter plus probability of it being CSF is exactly equal to 1.

1.6.5.4 Correction for volume changes (Jacobian modulation): As a result of spatial normalization, the volumes of certain cerebral regions (and their constituent voxels) will grow while others will shrink. For example, if a subject's amygdala was half the volume than the amygdala of the template image, then the volume of the subject's amygdala would be doubled during spatial normalization. Subsequent to image reslicing, this would result in the doubling of the number of voxels in the amygdala labelled as being grey matter. The challenge is how to preserve the volume of grey matter in the original image from the distortions arising from the spatial normalization procedure. The way in which SPM addresses this challenge is by multiplying the original segmented images with the Jacobian determinants of the spatial normalization (i.e. of each subject's original whole-brain image to the template).

The procedure of warping one image to another (e.g. a subject's image to a template), can be described by a 'deformation field' (Ashburner et al., 2000) that describes the transformations required for each voxel in the subject image in order to transform it to the corresponding voxel in the template image (Figure 1-6). It is possible to describe the amount of expansion or contraction required to warp a given voxel on the original image to the corresponding voxel on the template image by calculating the Jacobian determinant

of the specific voxel in the deformation field. The Jacobian determinant at a given voxel describes the determinant of the gradient of the deformation at this voxel (Freeborough & Fox, 1998). A Jacobian determinant of 2, for example, would correspond to the gradient of the deformation field describing a warp in which the volume **v** of given voxel **k** in the original image halved in volume to **v**/2 (Kipps et al., 2005). By multiplying the volume of **k** subsequent to warping (i.e. **v**/2) by the Jacobian determinant (i.e. 2) it is possible to calculate the volume of **k** in the original image (i.e. **v**). This procedure, known as 'Jacobian modulation' is the one employed in SPM.

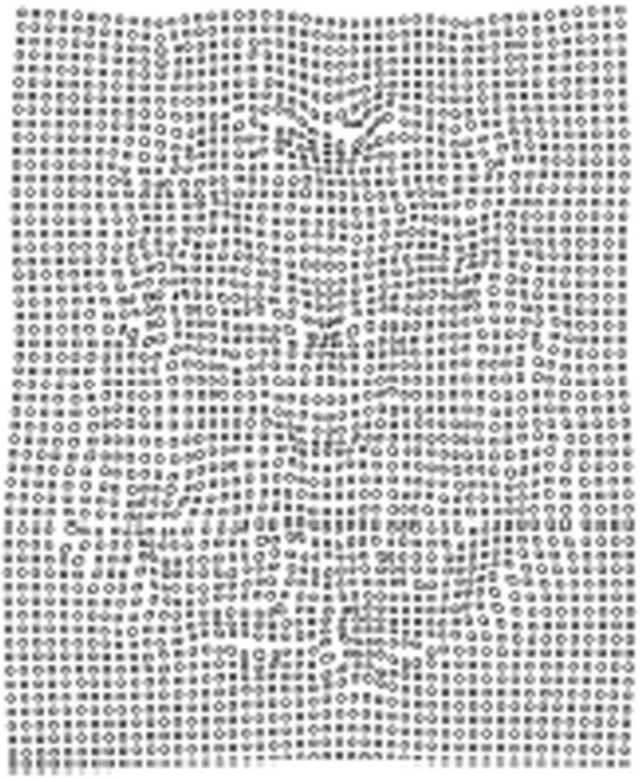

Figure 1-6: A pictorial representation of a deformation field describing the transformations necessary to warp the voxels (represented by the circles) of an example subject's image into the space defined by a template image. Image from Ashburner and Friston (2004)

Thus, in summary, if spatial normalization requires a subject's amygdala to double in size in order to match to the template, then the process of modulation involves halving the values of the constituent amygdala voxels for the purposes of calculating the volume of the amygdala in the original image.

It should be noted, though, that the physiological validity of equating the probability of a given voxel being grey matter with the volume of grey matter in that voxel has been questioned in the literature. A common criticism leveled at the SI techniques is that the measures derived from them are an abstraction of the imaging procedures employed rather than a true physical measure (such as millilitres for grey matter volume, or millimetres for cortical thickness), and as such are difficult to interpret with respect to the neuropathological literature.

1.6.5.5. Smoothing: The process of smoothing involves convolving each voxel in the normalized, segmented, cleaned and modulated images with a kernel defined by a 3-dimensional Gaussian curve. The size of the Gaussian kernel employed when smoothing is typically much larger than the voxel size of the image. For example, while a typical voxel size might be $1mm^3$, the full-width at half-maximum (FWHM) of a typical Gaussian used in VBM is 8 or 12mm, depending on anticipated size of the between-group differences being observed (Ashburner et al., 2000). Because of this, convolving a voxel **k** with a Gaussian has the effect of modifying the value of **k** based on the values of its surrounding voxels, with voxels more immediate to **k** given a heavier weighting. As Good et al. (2001) comments with regards to the smoothing of a pre-processed grey

matter image: "*the intensity of each voxel in the smoothed data is a locally weighted average of grey matter density from a region of surrounding voxels, the size of the region being defined by the size of the smoothing kernel*" (p.23). Figure 1-7 illustrates the effect of smoothing a series of black pixels with a Gaussian kernel. Note how convolution with the Gaussian 'softens' the sharp jumps in intensity of the original image, and transforms it from a series of discrete black points to a more continuous 'grey-scale' image.

Smoothing the pre-processed images with a Gaussian kernel renders the values of the voxels constituting the images more normally distributed, that is, it 'irons-out' sharp jumps in intensities between neighbouring voxels. This is very important, as a normal distribution of the data is a prerequisite for the parametric statistical analyses used in SPM, as described below. A second benefit of smoothing is that 'blurring' the images helps to compensate for the inexact nature of the spatial normalization (Ashburner et al., 2000).

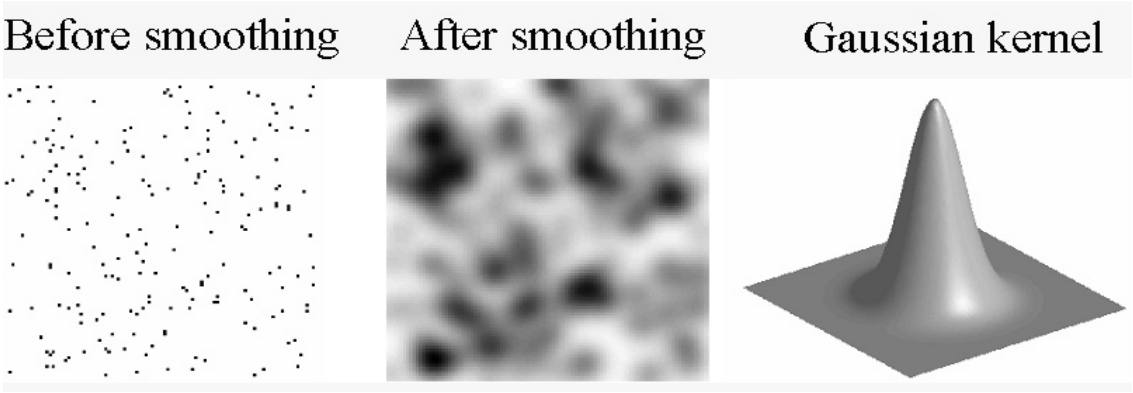

Figure 1-7: Smoothing an image with a Gaussian kernel – note how the image composed of discrete black pixels on the left is transformed into a continuous 'grey-scale' image on the right after convolution with a Gaussian kernel (from the Cognition and Brain Sciences Unit, Cambridge, UK; www.mrc-cbu.cam.ac.uk/Imaging/Common/rikSPM-preproc.ppt)

1.6.5.6 Statistical Analysis: The end products of the pre-processing stages are three normalized, cleaned, modulated, smoothed probability images (i.e. grey matter, white matter and CSF). As a result of the Jacobian modulation step, however, the value of voxel **k** can be interpreted as the *volume* of grey matter in voxel **k**. It is thus possible to imagine calculating the grey matter volume of voxel **k** for all control subjects and comparing these volumes to the volumes acquired for voxel **k** in the patients' images, or in other words, performing a *t*-test with diagnosis (patient or control) as the independent variable and grey matter volume for **k** as the dependent variable. This is essentially what is involved when examining the structural brain differences between patients with schizophrenia and matched healthy controls in SPM, except that a linear regression analysis is used rather than a *t*-test, with diagnosis being entered in as a dummy predictor variable (Figure 1-5). The advantage of using a linear regression analysis in this instance is that it is possible to statistically control for the effect of extraneous variables that may affect grey matter volume in and of themselves (e.g. age, gender), by including them as covariates of no-interest (nuisance covariates) in the model. Rather than limiting the statistical analysis to the between-group comparison of a single voxel (which would be akin to the tiniest possible ROI analysis!), SPM performs a statistical analysis at each and every voxel in the pre-processed MR images.

If a typical voxel size is $1mm^3$, then there are 1000 voxels per cubic centimetre of brain tissue. And given that the volume of the average human brain is approximately 1500 cubic centimetres (Grieve, Clark, Williams, Peduto, & Gordon, 2005), then it follows that there are approximately 1500 x 1000 = 1.5 million voxels in the average brain. As a

statistical analysis is performed at each voxel in the brain in VBM, this equates to approximately 1.5 million statistical analyses. This results in some serious issues regarding the inflation of the α-level due to the sheer number of statistical comparisons being made. This can be addressed in SPM either by a) statistically controlling the significance threshold for the effect of multiple comparisons, or b) using a highly stringent uncorrected significance threshold (usually $p<.0001$).

The procedure used to correct for multiple comparisons in SPM is based on the Gaussian Random Field (GRF) theory (Worsley, Evans, Marrett, & Neelin, 1992). The fundamental way in which the GRF correction procedure differs from the ubiquitous Bonferroni correction procedure (Bland & Altman, 1995), is that GRF theory recognizes that due to the images being smoothed, the voxel values of an image are not independent of each other and should not be considered as such for the purposes of statistical correction. Thus while a Bonferroni correction would control for the number of false-positive voxels, a GRF correction would control for the number of false positive regions. The number of regions controlled for in a GRF correction is dependent on the smoothness of the images analysed, as the value of a given voxel in a very smooth image (i.e. an image that was smoothed with a very large Gaussian kernel) will be dependent on the values of hundreds of its neighbouring voxels, and thus this image will require less correction than an image that has been smoothed with a very small Gaussian kernel (Friston, 2004).

I have now outlined the fundamentals of the two methods that have been most widely used to identify and quantify the neuroanatomical abnormalities present in patients with schizophrenia. I will now briefly review the findings of the previous MRI studies that have investigated for evidence of structural brain abnormalities in patients with both chronic and first-episode schizophrenia.

1.7 STRUCTURAL BRAIN ABNORMALITIES IN PATIENTS WITH SCHIZOPHRENIA

1.7.1 Structural brain abnormalities in patients with chronic schizophrenia

There have been numerous previous studies that have used MRI to investigate for evidence of structural brain abnormalities in patients with chronic schizophrenia, and both the ROI and SI methodologies have been widely used. The ROI studies in the literature have reported numerous brain structures to be volumetrically reduced in patients with chronic schizophrenia, including the prefrontal cortex (Buchanan, Vladar, Barta, & Pearlson, 1998), parietal cortex (Schlaepfer et al., 1994) , temporal cortex (Zipursky et al., 1994), medial temporal lobe limbic structures, such as the hippocampus and amygdala (DeLisi, Dauphinais, & Gershon, 1988), basal ganglia (Mion, Andreasen, Arndt, Swayze, & Cohen, 1991), thalamus (Andreasen et al., 1990), cerebellum (Okugawa et al., 2002) and corpus callosum (Gunther et al., 1991) – see reviews by Kasai et al. (2002), McCarley et al. (1999) and Pearlson et al. (1999). Most of these findings have been replicated by previous studies that have employed the statistical imaging

approach, with volumetric reductions in the frontal cortex (Sigmundsson et al., 2001), parietal cortex (Ananth et al., 2002), temporal cortex (Moorhead et al., 2004), medial temporal lobe limbic structures (Shapleske et al., 2002) , basal ganglia (Marcelis et al., 2003), thalamus (Hulshoff Pol et al., 2001), cerebellum (Marcelis et al., 2003) and corpus callosum (Hulshoff Pol et al., 2004) having been reported – see Honea et al. (2005) for a review.

There is a major confound, however, associated with studies that have investigated for evidence of neuroanatomical abnormalities in patients with chronic schizophrenia. This confound relates to the fact that patients with chronic schizophrenia have, almost without exception, undergone long-term exposure to various types of neuroleptic medication. This confound arises in light of evidence suggesting that long-term exposure to neuroleptic medication can alter brain structure in and of itself. For example, Nopoulos et al. (2001) reported a strong negative correlation between the volume of the midbrain and the cumulative exposure to typical neuroleptics (i.e. the phenothiazines (e.g. chlorpromazine), butyrophenones (e.g. haloperidol), diphenyl butylpiperidines (e.g. fluspiriline) and substituted benzamines (e.g. sulpiride) (Beaumont, 2000)) in 50 men with chronic schizophrenia. They suggested that the reduced midbrain volume resulted from a neuroleptic-induced state of tonic inactivation in midbrain dopamine activity, which is consistent with the trophic theory of neurodevelopment discussed previously (Purves, 1998). On the other hand, Gur et al. (1998c) reported that patients with schizophrenia who had experienced long-term exposure to typical neuroleptics exhibited *larger* volumes of the basal ganglia and thalamus than did neuroleptic-naïve patients and

healthy controls, while patients with long-term exposure to the atypical neuroleptics (e.g. risperidone and clozapine) exhibited larger thalamic volumes in comparison to the neuroleptic-naïve and control subjects. To complicate matters further, Corson et al. (1999) reported a positive correlation between basal ganglia volume and cumulative exposure to typical neuroleptics in patients with chronic schizophrenia, but a negative correlation between basal ganglia volume and cumulative exposure to atypical neuroleptics in patients with chronic schizophrenia

(As a side note, in order to calculate an estimate of patients' cumulative exposure to neuroleptic medication, it is necessary to convert their medication dosages into a common scale, of which the 'chlorpromazine-equivalent dosage' scale is the most popular. Tables for converting the dosages of various typical neuroleptics into the 'chlorpromazine-equivalent dosage' scale have been provided by Davis (1974), while conversion tables for the newer atypical neuroleptics have been provided by Herz (1997)).

Thus although the precise effect of chronic exposure to neuroleptic medication on brain structure is unclear (and may well be specific to the type of medication used), there is strong evidence to suggest that the chronic exposure to neuroleptic medication can influence patients' neuroanatomy in and of itself. This presents an obvious confound for studies that aim to identify the neuroanatomical underpinnings of schizophrenia. The most obvious way around this confound is to investigate patients who have had minimal exposure to neuroleptics. Given that the vast majority of patients with chronic

schizophrenia have had substantial exposure to antipsychotic medication (Kane, 1989), and given that it is not ethical or practicable withhold neuroleptic medication purely for the purposes of research, the most obvious way to minimise the confounding effects of neuroleptic exposure is to investigate patients with schizophrenia who have recently experienced their first psychotic episode. This 'first-episode' design, as it has been dubbed, has become increasingly popular in the psychiatric research literature, and it is the design that I employed for all of the empirical studies in this thesis.

The first-episode design also has a second benefit, which is related to the possibility that schizophrenia is a neurodegenerative disease. If progressive structural brain atrophy is, in fact, a feature of schizophrenia (and, as discussed in Section 1.5.3 there is significant evidence to suggest that it might be), then it is thus impossible when investigating patients with chronic schizophrenia to distinguish: 1) whether any observed abnormal volumetric reductions in regional brain tissue arose because of the disease itself or because of a chronic exposure to neuroleptic medications, and 2) whether the neuroanatomical abnormalities were present at the onset of the disease or whether they progressed neurodegeneratively over the course of the illness. By investigating the structural underpinnings of schizophrenia very early in the course of the disease, the first-episode design provides significant insight into the nature of the schizophrenia, its origins, its clinical course and the optimal path for therapeutic intervention, while minimizing the confounds associated with the investigations of patients in the chronic phase of the disease.

1.7.2 Structural brain abnormalities in patients with first-episode schizophrenia

Compared to the large amount of research that has been conducted on patients with chronic schizophrenia, there have been relatively few studies that have investigated for evidence of structural brain abnormalities in patients suffering from their first episode of schizophrenia (FES). The results of the studies that have been undertaken are somewhat equivocal.

On the one hand, there have been a number of studies that have reported structural brain abnormalities in patients with FES. For example, Hirayasu et al. (2001) employed a ROI design to compare the grey and white matter volumes of the PFC between 17 FES patients and 17 control subjects, and reported lower GM volumes in the PFC in the FES patients.

Joyal et al. (2002) observed 13 neuroleptic-naïve to exhibit reduced volumes of the manually-defined entorhinal cortex (i.e. the medial aspect of the parahippocampal gyrus) bilaterally, compared to 22 control subjects. In the same subject sample, Joyal et al. (2003) also observed the FES patients to exhibit abnormally reduced volumes of the amygdala bilaterally.

Diwadkar et al. (2004) used the signal intensity of T1-weighted MR images as the dependent variable in their study which investigated the differences in the structural

integrity of the corpus callosum between 29 FES patients and 62 healthy controls. Diwadkar et al. (2004) reported reduced signal intensities in the genu, body, isthmus and splenium of the corpus callosum in the FES patients. They argued that this reduced signal intensity reflected an abnormally elevated concentration of water in the characteristically fatty tissue of the corpus callosum which, they argued, was due either to abnormalities in the microtubular densities of the axonal cytoarchitecture, abnormalities in myelination, or alterations in the density of white matter fibres.

Hirayasu et al. (1998) investigated the volumetric GM differences between 17 FES patients and 18 matched controls in three manually-defined ROIs: the superior temporal gyrus, amygdala-hippocampal complex, and parahippocampal gyrus. They identified volumetric reductions in the GM of the left superior temporal gyrus and left amygdala-hippocampal complex in the FES patients. Furthermore, consistent with Crow's (1997) theory (Section 1.5.6), Hirayasu et al. (1998) also identified evidence of abnormal cerebral asymmetry in the FES patients. They reported patients with FES to exhibit a significant 'left-less-than-right' asymmetry in the superior temporal gyrus and amygdala-hippocampal complex that was not present in the control subjects.

Job et al. (2002) were one of the first groups to use the technique of voxel-based morphometry (VBM) in SPM to investigate for evidence of neuroanatomical abnormalities in patients with FES. Using a voxel-level probability threshold (known as the 'height threshold') of $p<.05$ (corrected for multiple comparisons using GRF theory; see Section 1.6.5.6), Job et al. (2002) observed reduced GM volumes in the right frontal

lobe (anterior cingulate and medial frontal gyrus), left middle temporal gyrus, left postcentral gyrus and left amygdale, in 34 FES patients relative to 36 matched controls. These results were, by in large, consistent with the results of the study by Jayakumar et al. (2005). Jayakumar et al. (2005) also used VBM in SPM to identify, on a voxel-by-voxel basis, the regions of volumetric GM abnormality present in 18 neuroleptic-naïve, FES patients, compared to 18 matched healthy controls. They reported the FES patients to exhibit reduced GM volumes in the cingulate cortex, superior frontal gyrus, post-central gyrus, parahippocampal gyrus, inferior parietal lobule, thalamus, caudate, language centres in the inferior frontal gyrus and superior temporal gyrus and, consistent with the predictions of Andreasen (1999), in the cerebellum.

In contrast to these VBM studies that have reported widespread GM deficits in patients with FES, Kubicki et al. (2002) used VBM in SPM to investigate for GM abnormalities in 16 FES patients relative to 18 healthy controls, and only observed GM 'density'[1] reductions in a small portion of the left superior temporal gyrus when correcting for multiple comparisons using GRF theory.

Salgado-Pineda et al. (2003) also used VBM in SPM to identify abnormalities in GM 'density' in 13 male, neuroleptic-naïve FES patients compared to 13 matched healthy controls. When employing a height-threshold of $p<.001$ (uncorrected for multiple comparisons) and a required minimum cluster-size of 20 voxels (known as the 'extent

[1] In this context, the GM 'density' of a voxel refers to its probability value after normalization, segmentation and smoothing, i.e. without modulation with the Jacobian determinants derived from the spatial normalization. As explained in the paper by Good et al. (2001), *"an analysis of modulated data tests for regional differences in the absolute amount (volume) of grey matter, whereas analysis of unmodulated data tests in concentration of grey matter (per unit volume in native space)"* (p.24).

threshold'), Salgado-Pineda et al. (2003) reported the FES patients to exhibit decreased GM 'density' in the anterior cingulate gyrus, left inferior frontal gyrus, thalamus, caudate, left hippocampus and left parahippocampal gyrus, relative to the control subjects. However, in addition to this, Salgado-Pineda et al. (2003) also reported the FES patients to exhibit abnormally *increased* 'densities' in the left superior temporal gyrus, left insular cortex, left putamen and right supramarginal gyrus. These observations of regional 'density' increases in the FES patients is consistent with the proposal of Feinberg (1982), who first raised the possibility of 'too few' synapses being eliminated in patients with schizophrenia, and argued that such an 'under-prune' could be just as damaging as the 'over-prune' that has been argued to underlie the characteristic GM reductions in schizophrenia (Keshavan, Anderson, & Pettegrew, 1994).

It is clear that there is a considerable degree of variation in the extent and spatial location of the neuroanatomical abnormalities that have been reported in patients with FES. Furthermore, several other studies have failed to observe any evidence of structural brain abnormalities in patients with FES. For example, Laakso et al. (2001) failed to observe any abnormality in the volume of the hippocampus in 22 neuroleptic-naïve FES patients compared to 18 control subjects, using an ROI approach. Lang et al. (2001) manually calculated the volumes of caudate, putamen and globus pallidus in 30 FES patients and 23 controls, and failed to find any between-group differences. This result is consistent with the results of the study by Gunduz et al. (2002), who reported no differences in the volumes of selected, manually-defined basal ganglia structures (specifically, the subcommisural limbic forebrain, nucleus accumbens, caudate and putamen). It is also

consistent with the results of the study by Gur et al. (1998b), who did not report volumetric deficits in the caudate, putamen and globus pallidus in 21 neuroleptic-naïve FES patients compared to 128 comparison subjects, but did report volumetric deficits in the thalamus. The failure to observe volumetric GM reductions in the basal ganglia are, however, inconsistent with a number of other studies (e.g. Jayakumar, Venkatasubramanian, Gangadhar, Janakiramaiah, & Keshavan, 2005; Salgado-Pineda et al., 2003). Finally, using an ROI approach, Niemann et al. (2000) failed to observe structural brain abnormalities in the temporal lobe, amygdala and hippocampus in 20 male FES patients, compared to 20 matched healthy controls, despite the fact that each of these regions have been among the most consistently implicated in the literature as being structurally abnormal in patients with FES.

Thus it is clear that in spite of the efforts of the relatively small number of previous studies, a definitive answer as to what specific neuroanatomical abnormalities underlie first-episode schizophrenia remains elusive. The relatively small patient samples (generally <30) that have typically been used in previous studies could, at least in part, account for these inconsistent results. Furthermore, very few studies have explored the intimately related issues of whether these structural brain abnormalities are static or progressive over the first few years of patients' illness, and whether they have identifiable clinical and neurophysiological correlates.

Identifying the neuroanatomical abnormalities associated with FES in a larger patient sample, and determining whether these abnormalities were static or progressive over the

first few years of patients' illness, were the primary aims of this thesis. Furthermore, this thesis also aimed at identifying the clinical and neurophysiological correlates of these structural brain abnormalities in the FES patients. I will expand upon these aims in the following section, and I will also discuss how these aims were addressed in the subsequent empirical chapters.

1.8 GENERAL AIMS OF THE THESIS

Having discussed the symptomatology of schizophrenia, theories as to its aetiology and neuroanatomical underpinnings, the development of the neuroimaging and associated analysis techniques that provide a method of validating or disproving these theories, and a review of the previous studies that have investigated for evidence of neuroanatomical abnormalities in patients with both chronic and first-episode schizophrenia, it is now finally possible to state the general aims of this thesis:

The first aim of this thesis, addressed in Chapter 2, was to use structural MRI to identify and quantify the neuroanatomical changes that occurred over adolescence and young adulthood in healthy people. As discussed in Section 1.5.4, the fact that schizophrenia so frequently first presents in the period between late adolescence and early adulthood illustrates the importance of identifying the structural and functional brain changes associated with this period, and of elucidating the mechanisms underlying these transformations.

The second, and in many ways primary aim of this thesis was to identify and quantify the regional differences in grey matter volume (Chapter 3) and white matter volume (Chapter 4) between patients experiencing their first-episode of schizophrenia (FES) and matched healthy controls, both at the time of patients' first presentation to mental health services with psychotic symptoms (baseline condition) and 2-3 years subsequently (follow-up condition). In other words, the studies described in these two chapters aimed to identify the grey and white matter abnormalities that were present in patients with schizophrenia around the time of their first psychotic episode, and also to identify whether there was any evidence of neurodegeneration occurring over the first 2-3 years of illness in these patients, over and above any progressive tissue loss due to normal maturational processes.

The third aim of this thesis, addressed in Chapter 5, was to identify the neuroanatomical correlates of FES patients' symptomatology. Specifically, Chapter 5 aimed at identifying whether FES patients characterised by a certain clinical profile (e.g. severe Psychomotor Poverty) exhibited differences in brain structure compared to FES patients characterised by a different clinical profile (e.g. severe Reality Distortion). This issue has important implications with respect to theories that have argued that rather than being a unified disorder, schizophrenia should instead be considered as a number of discrete disorders. The historical distinction between hebrephrenic, paranoid and undifferentiated schizophrenia (Fenton & McGlashan, 1991) is an example of such a conceptualisation. Furthermore, by identifying the nature of the relationship between patients' brain atrophy

and their symptomatology, it was hoped to be able to better elucidate the neurological origins of the various symptoms of schizophrenia.

The fourth and final aim of this thesis was to identify the relationship between the age-related changes in brain structure and the age-related changes in brain electrophysiology (assessed with electroencephalography; EEG) in healthy adolescents/ young adults (Chapter 2) and FES patients (Chapter 6). The rationale behind this aim was that the identification of an abnormal relationship between brain structure and function in patients with FES could provide an insight into the nature of the dysfunctional neural connectivity that has been so widely proposed to underlie schizophrenia (Andreasen, 1999; Crow, 1997; Frith, 1992).

Two closely related studies, which were included as Appendices 1 and 2, investigated the neuropsychological correlates of longitudinal brain change in patients with FES, and examined how specific the observed neuroanatomical abnormalities were to schizophrenia, in comparison to the other major psychotic illness of bipolar disorder. Although these two studies were not directly relevant to the aims outlined above, they provided further insight into the nature of the neuroanatomical underpinnings of schizophrenia, and thus were included as supplementary material.

CHAPTER 2

BRAIN MATURATION IN ADOLESCENCE: CONCURRENT CHANGES IN NEUROANATOMY AND NEUROPHYSIOLOGY

2.1 PREAMBLE

In light of theories such as Feinberg's (1982), which have emphasized the role of dysfunctional adolescent brain development in the aetiology of schizophrenia, the primary aim of Chapter 2 was to use structural MRI to identify and quantify the neuroanatomical changes associated with adolescence and early adulthood in a large group of healthy participants. To this end, MR images were collected from 138 healthy participants aged between 10 and 30 years. The MR images were pre-processed using SPM before being segmented into four grey matter and four white matter regions corresponding to the four lobes of the brain (i.e. the frontal, temporal, parietal and occipital lobes). The volumes of these regions were then calculated, which allowed for the examination of age-related trends in regional grey and white matter volumes.

The secondary aim of this study was to investigate what relationship, if any, existed between the age-related changes in subjects' regional grey matter volumes and the age-related changes in brain electrophysiology in corresponding cortical regions. To this end, electroencephalographic (EEG) scans were collected from all subjects, and absolute EEG power scores were calculated for three frequency bands (slow-wave, alpha and beta) across four large-scale cortical regions that corresponded roughly to the four brain lobes examined in the MRI analysis. This allowed for the examination of age-related trends in regional absolute EEG power, and the subsequent comparison between these EEG changes and the associated MRI changes in the corresponding cortical regions. The rationale for identifying the relationship between brain structure and brain

electrophysiology in healthy participants was that it would provide a reference for the exploration of this same relationship in patients with first-episode schizophrenia. This subsequent investigation formed the basis of Chapter 6.

The research described in this chapter has been published in the journal *Human Brain Mapping* as an article entitled "Brain maturation in adolescence: concurrent changes in neuroanatomy and neurophysiology" (see Whitford et al., 2007c and Appendix 5).

2.2 ABSTRACT

Adolescence to early adulthood is a period of dramatic transformation in the healthy human brain, however the relationship between the concurrent structural and functional changes remains unclear. This paper investigated the impact of age on both neuroanatomy and neurophysiology in the same healthy subjects (n=138) aged 10 to 30 years, using magnetic resonance imaging (MRI) and resting electroencephalography (EEG) recordings. MRI data were segmented into grey and white matter images and parcellated into large-scale regions-of-interest. Absolute EEG power was quantified for each lobe for the slow-wave, alpha and beta frequency bands. Grey matter volume was found to decrease across the age bracket in the frontal and parietal cortices, with the greatest change occurring in adolescence. EEG activity, particularly in the slow-wave band, showed a similar curvilinear decline to GM volume in corresponding cortical regions. An inverse pattern of curvilinearly increasing white matter volume was observed in the parietal lobe, which suggested that the reduction in grey matter primarily reflected

a reduction of neuropil, and that the corresponding elimination of active synapses was responsible for the observed reduction in EEG power.

2.3 INTRODUCTION

Whilst the most dramatic structural changes in the healthy human brain are thought to occur in the perinatal period (Huttenlocher & Dabholkar, 1997), there is a growing body of evidence suggesting that adolescence is also a period of substantial neurodevelopment (Sisk & Foster, 2004). Research into brain maturation over adolescence and early adulthood is particularly important, given that it is a peak period of neural reorganization which contributes to both normal variation and the onset of major mental illnesses, such as schizophrenia (Keshavan et al., 1994). Despite growing evidence for pronounced changes in both the structure and function of the brain during adolescence and early adulthood, the relationship between these changes has not been directly examined. The objective of this study was to examine these concurrent developmental changes by obtaining both neuroanatomical measures of structural change (with magnetic resonance imaging, MRI) and electroencephalographic indices of neural function, for the first time in a large sample (n=138) of healthy subjects.

A number of MRI studies have reported an intense period of grey matter tissue loss in the peripubescent period between 10 and 18 years of age (Giedd et al., 1999; Pfefferbaum et al., 1994; Steen, Ogg, Reddick, & Kingsley, 1997). This grey matter loss, which appears to occur most severely in the association cortices (Sowell et al., 1999), is thought not to

be primarily due to neuron death, but rather due to the elimination of naturally overproduced synapses and their associated neuropil (dendrites, dendritic spines and axon terminals) (Purves, 1998). Increased competition for trophic factors in target cells, possibly mediated by hormonal influences, has been proposed as a cause for this 'synaptic prune' (Purves et al., 1980). Regarding white matter, previous studies have reported increases in white matter volume over the peripubescent period, especially in the frontal lobe and hippocampal relays (Benes, Turtle, Khan, & Farol, 1994; Benes, 1989). This white matter gain, which is thought to reflect increased axonal myelination (Paus et al., 2001), has been associated with the development of language and memory skills in adolescence (Nagy, Westerberg, & Klingberg, 2005).

Complementing the neuroanatomical studies, changes in brain function during adolescence have been investigated by electrophysiological means. Matousek and Petersen (1973), for example, measured changes in the electroencephalogram (EEG) in a large group (n = 348) of adolescents/young adults aged 10 to 20 years and found that absolute EEG power decreased in all frequency bands, particularly in the slow-wave band of below 7.5 Hz. This finding has since been replicated (Gasser, Verleger, Bacher, & Sroka, 1988; Matsuura et al., 1985). This decrease has been found to continue into adulthood, albeit at a lesser rate than in adolescence (Dustman, Shearer, & Emmerson, 1999).

The EEG signal is thought to arise from areas of spatially coherent synaptic activity at the cortex (Niedermeyer & Lopes de Silva, 1999), with absolute EEG power being related to

the amplitudes of the resultant 'waves' in scalp electrical potential. It might be expected, therefore, that a dramatic reduction in the number of cortical synapses in adolescence would correspond to a substantial reduction in absolute EEG power. While this relationship has not been explicitly examined, Feinberg (1982) alluded to the idea when he noted that Huttenlocher's (1979) data of synaptic density in the cerebral cortex showed a similar trend across age to his own data on slow wave EEG power in sleep, with both showing a steep reduction in early adolescence. Evidence for a grey matter – EEG relationship, independent of age, comes from head injured patients with grey matter atrophy who show a corresponding decrease in EEG amplitude, especially in the alpha and beta bands (Thatcher, Biver, McAlaster, Camacho, & Salazar, 1998). However no previous studies (to my knowledge) have directly investigated the relationship between the anatomical and electrophysiological brain changes that occur in adolescence and early adulthood.

In this study, both structural MRI and concurrent quantified EEG were examined in healthy subjects aged between 10 and 30 years. It was anticipated that subjects' grey matter volume would *decrease* curvilinearly over this period, especially in the frontal and parietal regions, reflecting the extended maturation of the association cortices. A corresponding decrease in absolute power was expected for the EEG data in the corresponding cortical regions. On the other hand, it was hypothesised that white matter volume would *increase* curvilinearly across this period, again most apparently in the frontal and parietal lobes. It was expected that the greatest differences in both

neuroanatomy and neurophysiology would be apparent during adolescence before reaching an asymptote in the mid-twenties.

2.4 METHODS

2.4.1 Participants

138 healthy participants (70 female, 68 male), distributed evenly between 10 and 30 years of age (see Table 2-1) were recruited from the Brain Resource International Database (BRID; http://www.brainresource.com). Exclusion criteria included a personal history of mental illness, physical brain injury, neurological disorder or other serious medical condition and/or a personal history of drug or alcohol addiction. The SPHERE questionnaire (Hickie et al., 1998) was used to screen for likely Axis-1 psychiatric disorder. All subjects (or their guardians for subjects less than 18 years of age) provided written informed consent to participate in the database. Subjects were required to refrain from caffeine intake and from smoking for at least two hours prior to the EEG testing.

	Age-bracket (years)						
	10-14	**15-17**	**18-19**	**20-21**	**22-24**	**25-29**	**Total**
# Males	8	8	11	11	12	20	70
# Females	11	11	11	7	12	16	68
Total	19	19	22	18	24	36	138

Table 2-1: Breakdown of the subject sample (n=138) in terms of age and gender

2.4.2 MR imaging and parcellation

All subjects underwent a single T1-weighted volumetric MPRAGE structural MRI scan on a Siemens 1.5 Tesla Vision Plus system at Westmead Hospital, Sydney. Images were obtained in the sagittal plane, with scan parameters: TR = 9.7msec, TE = 4msec, TI = 200msec, flip angle = 12°. A total of 180 contiguous 1 mm slices were acquired with a 256 x 256 matrix with an in plane resolution of 1 mm x 1 mm, resulting in isotropic voxels.

Images were processed using SPM2 (Wellcome Department of Cognitive Neurology, London, UK), running on Matlab 6.5 (MathWorks, Natick, USA). The full details of the processing protocol used in voxel-based morphometry (VBM) are presented elsewhere (Ashburner et al., 2000; Good et al., 2001). Briefly, subject brain images were firstly spatially normalized by transforming each brain into a standardized stereotactic space based on the ICBM 152 template (Montreal Neurological Institute), which approximated Talairach space. This process was performed using a custom T1-image template created from 333 brain images from the BRID that were acquired using the same protocol. The first step in spatial normalization involved estimating the optimum 12-parameter affine transformation (3 translations, 3 rotations, 3 zooms and 3 shears) for matching the subject's image to the template. The second step accounted for global non-linear shape differences, which were modelled by a linear combination (7x8x7) of smooth spatial basis functions (Ashburner et al., 2000). The normalized images were re-sliced with 1.5 x.1.5 x 1.5 mm voxels, before being segmented into grey matter (GM), white matter

(WM) and cerebrospinal fluid (CSF) probability maps, and stripped of extra-cerebral voxels. Segmentation was based on a cluster analysis method that accounted for each voxel's signal intensity, together with an *a priori* expectation of the anatomical location of the different tissue types. In order to adjust for the growth and shrinkage of voxels that can occur during spatial normalisation, voxel probability values in the cleaned, segmented images were modulated with the Jacobian determinants derived from the spatial normalization (Good et al., 2001). Thus if a brain region doubled in size as a result of normalization, the grey matter probability value for this region would be halved for the purposes of calculating its volume (Ashburner & Friston, 2001). The processed GM, WM and CSF images were smoothed with a Gaussian kernel of 12mm full-width at half-maximum, prior to volume calculation.

The pre-processed grey and white matter images were then automatically parcellated into regions-of-interest (ROIs). Five GM supraregions (frontal, parietal, temporal, occipital and limbic lobes) were parcellated using the previously published Automatic Anatomical Labelling (AAL) masks (Tzourio-Mazoyer et al., 2002) as listed in Table 2-2 and illustrated in Figure 2-1. The AAL parcellation was chosen as the basis for the segmentation because of its definition in MNI space, which is a standardized co-ordinate system in common usage. Parcellation was performed in an axial view, and assignments were cross-checked with AAL parcellation to ensure consistency. The frontal lobe was defined as all cerebrum anterior to the central sulcus and superior to the Sylvian fissure. At the deepest point of the central sulcus a straight line was drawn to the interhemisphereic fissure. In planes where the anterior horns of the lateral ventricles were

visible, a line was drawn from the central sulcus to the anterior limit of the ventricles, then back to the interhemisphereic fissure. The corpus callosum was separately outlined to exclude it from the frontal lobe. In slices containing the insula, with reference to the AAL parcellation, this region was excluded, and a line was drawn from the Sylvian fissure to the interhemisphereic fissure. Inferior regions of the brain were clearly separated from temporal lobe structures by CSF. The parietal lobe was traced in a sagittal plane defined as all cerebrum superior and anterior to the parieto-occipital sulcus, posterior to the central sulcus, and superior to the corpus callosum. The occipital lobe was defined as everything posterior to the boundaries described below. Parcellation of the occipital lobe was performed from the midline in a para-sagittal view. The antero-superior border was defined by the parieto-occipital and the temporo-occiptal sulci. Lateral to the midline, from where the parieto-occipital sulcus was no longer prominent, a straight line was drawn between it and the horizontal ramus of the superior temporal sulcus. The postero-inferior border was defined by the anterior calcarine sulcus, the collateral sulcus and the posterior transverse collateral sulcus and included the lingual gyrus. The temporal lobe included the superior, middle and inferior gyri. The borders were defined by the Sylvian fissure, occipitotemporal sulcus, superior temporal sulcus and the lateral border of the parahippocampal gyrus. The limbic lobe was created by amalgamating the following discrete structures: cingulate and paracingulate gyri, amygdala, hippocampus and parahippocampal gyrus.

Grey matter supraregions and their constituent AAL masks	
Frontal Lobe	**Limbic Lobe**
Precentral gyrus	Anterior cingulate and paracingulate gyri
Superior frontal gyrus, dorsolateral	Median cingulate and paracingulate gyri
Superior frontal gyrus, medial	Posterior cingulate gyrus
Superior frontal gyrus, medial orbital	Hippocampus
Superior frontal gyrus, orbital part	Parahippocampal gyrus
Middle frontal gyrus, orbital part	Amygdala
Middle frontal gyrus	
Inferior frontal gyrus, opercular part	**Occipital Lobe**
Inferior frontal gyrus, triangular part	Cuneus
Inferior frontal gyrus, orbital part	Lingual gyrus
Rolandic operculum	Superior occipital gyrus
Paracentral lobule	Middle occipital gyrus
Supplementary motor area	Inferior occipital gyrus
Olfactory cortex	Fusiform gyrus
Gyrus rectus	Calcarine fissure and surrounding cortex
Temporal Lobe	**Parietal Lobe**
Superior temporal gyrus	Postcentral gyrus
Temporal pole: superior temporal gyrus	Superior parietal gyrus
Middle temporal gyrus	Inferior parietal, minus supramarginal and angular gyri
Temporal pole: middle temporal gyrus	Supramarginal gyrus
Inferior temporal gyrus	Angular gyrus
Heschl gyrus	Precuneus

Table 2-2: The five supraregional grey matter masks and their constituent Automatic Anatomical Labelling (AAL) masks

Very few neuroanatomical studies have examined regional WM volumes, due to the difficulty in distinguishing between various WM tracts when manually defining ROIs. An advantage of using a common set of masks for all subjects is that it is unbiased, and ensures that the region of brain defined by the mask is consistent between subjects, assuming that global differences in brain shape have been largely removed by spatial normalization. The four WM masks used in this study are displayed in Figure 2-1. The

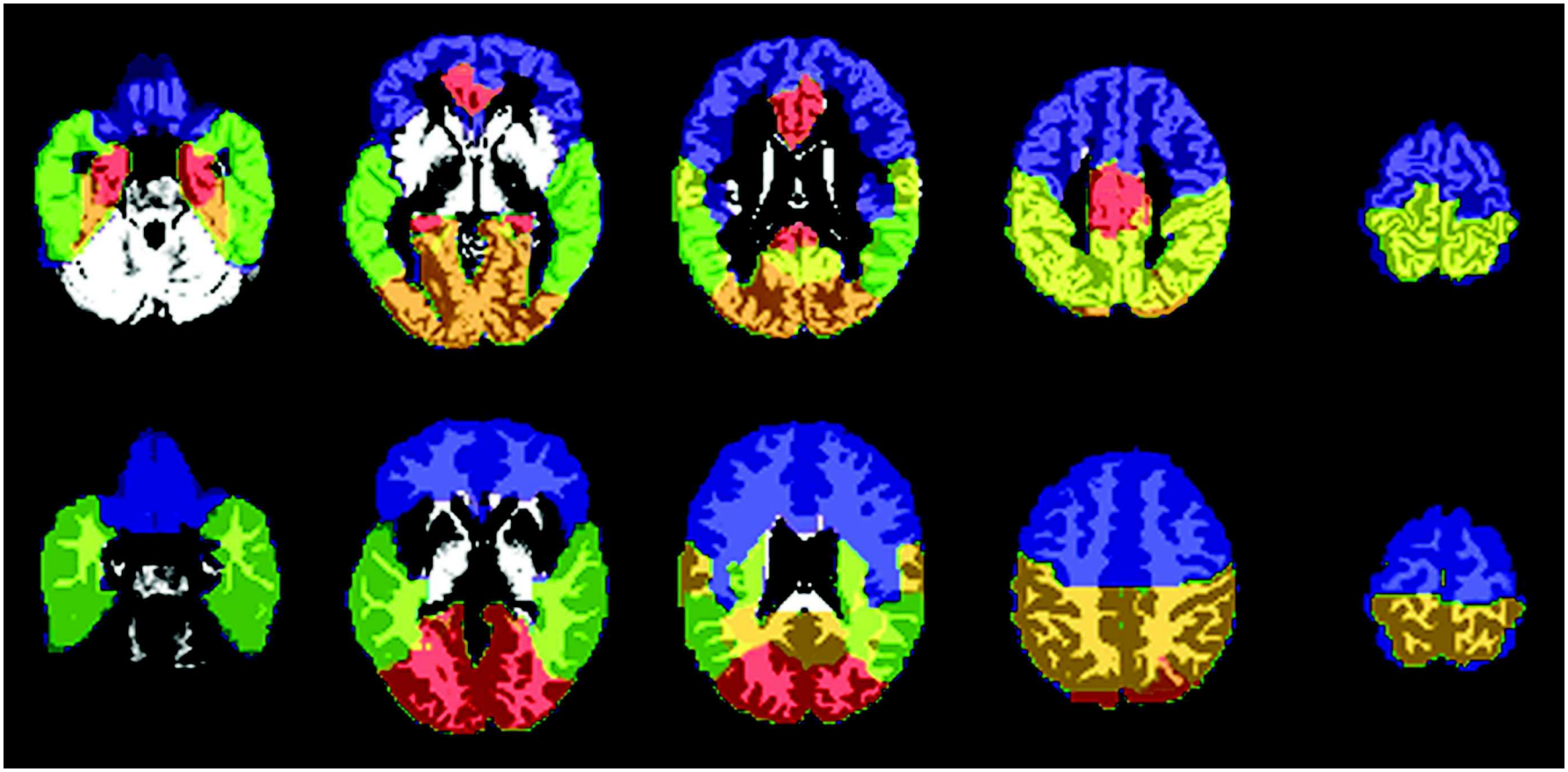

Figure 2-1: Illustration of the regions-of-interest identified for the structural MR images. The five grey matter regions-of-interest are shown on top, superimposed on the GM segment of the MNI T1-weighted single-subject brain (blue = frontal, green = temporal, orange = occipital, yellow = parietal, red = limbic). The four white matter regions-of-interest are shown below, superimposed on the WM segment of the MNI single-subject image (blue = frontal, green = temporal, orange = parietal, red = parietal

WM ROIs were generated in MNI space, in consultation with a radiologist, and were defined with reference to the AAL model and a segmented WM image as follows. The corpus callosum and internal capsule were defined first, the external borders of these being used to limit the internal aspects of the frontal, parietal and temporal lobes. The occipito-parietal border was interpolated from the borders of the superficial occipital and parietal lobes to the cuneus-precuneus border. The temporo-occipital border was similarly defined by the previously parcellated borders of the occipital and temporal lobes. The frontal lobe border followed the central sulcus superficially, with the internal border defined as anterior to the putamen and caudate at the level of the anterior commisure, and as anterior to the amygdala inferiorly.

Volumes for the five GM and four WM regions were calculated by summing the values of the constituent voxels following Jacobian modulation.

2.4.3 Electrophysiological data acquisition and parcellation

EEG was acquired using an electrode cap ("Quikcap", Neuroscan, USA) with 28 sites using the 10-20 system of electrode placement. Horizontal eye movements were recorded with electrodes placed 1.5 cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3 mm above the middle of the left eyebrow and 1.5 cm below the middle of the left bottom eye-lid. The impedance at each site was below 10 kOhms. Data were obtained continuously using a NuAmps system (Neuroscan, USA), a DC amplifier with large dynamic range (±132 mV and 22 bit

resolution). Each channel was sampled at 500 Hz after low pass filtering with attenuation of 40 dB per decade above 100 Hz. Data were corrected for eye movement offline (Gratton, Coles, & Donchin, 1983), and re-referenced to the average of A1 and A2 (mastoids). The EEG data reported and analysed in the present study were recorded during a two-minute interval in which subjects were asked to rest quietly with their eyes closed.

Spectral power estimation was performed by first re-sampling the data at 512 Hz, before applying a Welch window to each successive 4 second epoch and then performing a fast Fourier transform (FFT). The resultant power spectra were averaged separately for each electrode. Power (in μV^2) was then calculated for three frequency bands - slow-wave (0.5-7.5 Hz), alpha (8-12 Hz) and beta (12.5-34.5 Hz) - and square-root transformed in order that the values approximated the normal distribution required by parametric statistical methods.

To investigate the relationship between the change in neuroanatomical grey matter volume and the corresponding change in neural activity, four large-scale neural regions were created ('EEG regions') that corresponded to the four cortical GM regions. Neural activity over the frontal, parietal, temporal and occipital lobes was calculated by averaging the standardized power recorded at each electrode site contributing to each region, for slow-wave, alpha and beta activity. For example, frontal lobe power for the alpha band was the average of standardized alpha power recorded from Fp1, Fp2, F7, F3, FZ, F4, F8, FC3, FCZ and FC4 electrode sites. A schematic diagram of the electrodes constituting the four 'EEG regions' is provided in Figure 2-2.

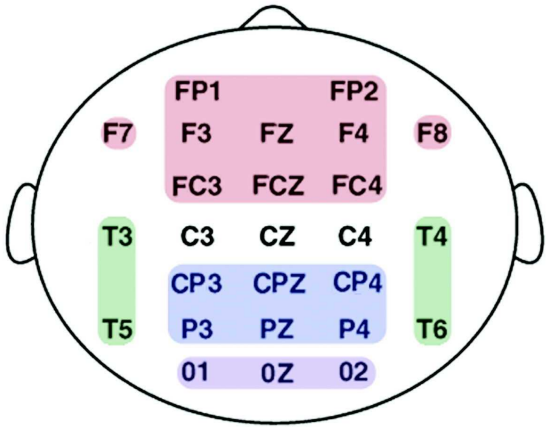

Figure 2-2: Schematic diagram of the constituent electrodes of the four 'EEG regions'. Electrodes making up the frontal lobe 'EEG region' are in red, the temporal lobe 'EEG region' in green, the parietal lobe 'EEG region' in blue and the occipital lobe 'EEG region' in purple. The average power of each 'EEG region' (for each frequency band for each subject) was calculated by averaging the standardized absolute power scores of the constituent electrodes.

2.4.4 Statistical Analysis

Linear regression analyses were used to assess the age-related trends in GM and WM volume, and in absolute power for the corresponding 'EEG regions'. As it was anticipated that the rate of structural and functional brain change would be highest in adolescence before decreasing in the twenties, subjects' ages were logarithmically transformed before being entered as the predictor variable-of-interest. If the regression model was found to account for significant variance in the response variable (e.g. GM volume), the significance of the standardized regression coefficient (β) for the predictor variable (log (age)) was considered. Partial correlation analyses were used to assess the

linear relationship between subjects' regional GM volumes and the averaged absolute power of their corresponding 'EEG regions'. Due to the number of statistical comparisons made, an α level of .01 was used as the threshold for significance for all analyses. Gender was statistically controlled for in all analyses.

2.5 RESULTS

Grey matter volume was found to reduce linearly with the log of age in the frontal and parietal lobe supraregions. The log of age was not found to be a significant predictor of tissue volume in the temporal, occipital or limbic GM supraregions (see Table 2-3 and Figure 2-3).

MRI Supraregion	β log (age)	t (β)	Sig (t)	R^2 change log(age)	R^2 model	F (model)	Sig (model)
Grey Matter Raw							
Frontal Lobe	-.229	-3.117	.002	.052	.280	26.29	<.001
Temporal Lobe	-.110	-1.584	.115	.012	.359	37.78	<.001
Parietal Lobe	-.227	-3.088	.002	.051	.281	26.35	<.001
Occipital Lobe	-.088	-1.205	.230	.008	.282	26.51	<.001
Limbic Lobe	-.088	-1.252	.213	.008	.333	33.64	<.001
Subcortical	-.089	-1.262	.209	.008	.336	34.14	<.001
White Matter Raw							
Frontal Lobe	.194	2.451	.016	.037	.187	15.05	<.001
Parietal Lobe	.214	2.808	.006	.046	.244	21.15	<.001
Occipital Lobe	.173	2.340	.021	.030	.285	26.06	<.001
Temporal Lobe	.195	2.594	.011	.038	.260	23.06	<.001

Table 2-3: Linear regression analyses for the MR data, controlling for gender. Model: Tissue Volume = b0 + b1(gender) + b2(log(age)).

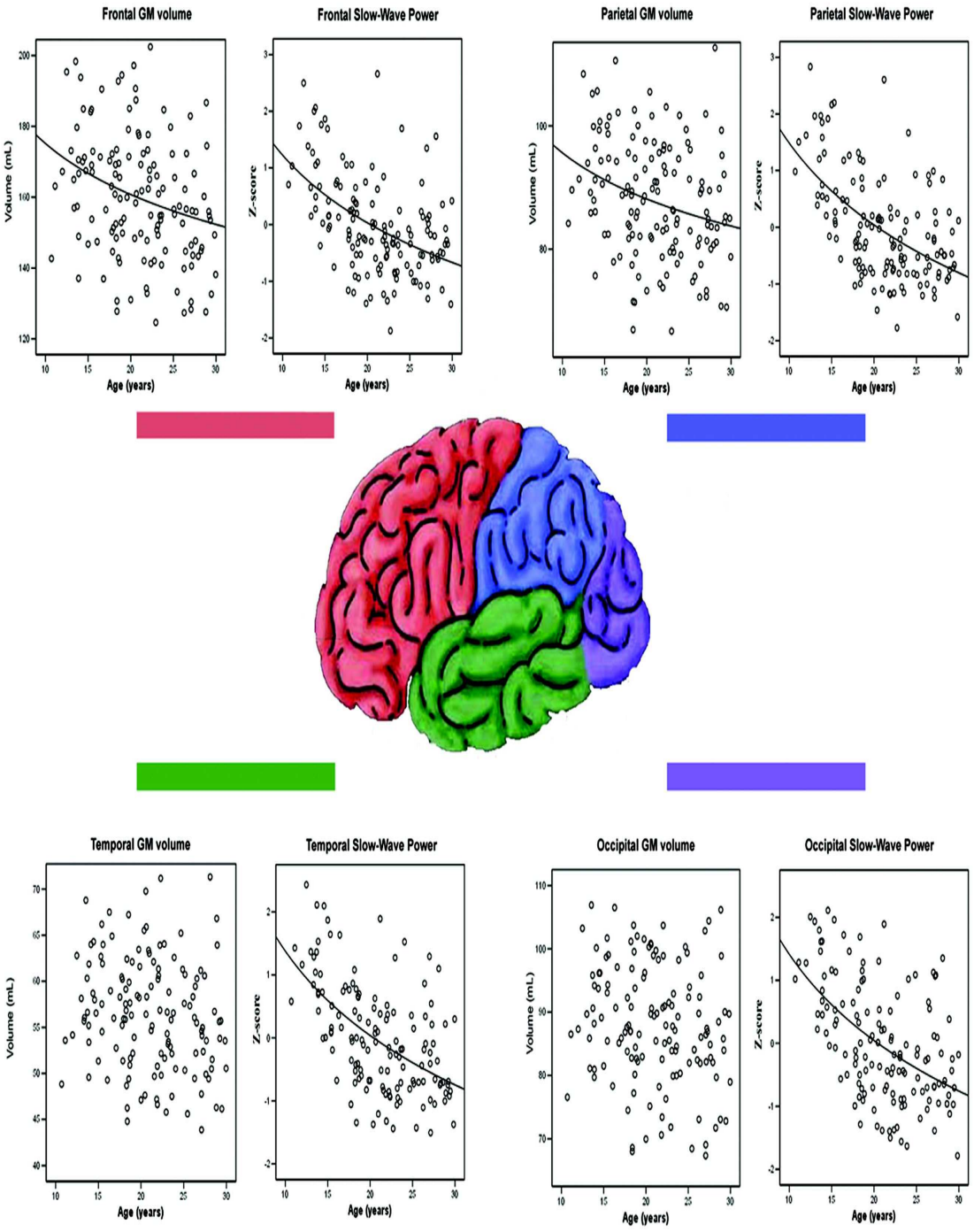

Figure 2-3: Grey matter vs age scatterplots for the frontal, parietal, temporal and occipital regions-of-interet

Absolute EEG power was also found to decrease linearly with the log of age for each of the three frequency bands across each of the four cortical 'EEG regions' i.e. frontal, temporal, parietal and occipital lobe. The regression model was found to fit the age-related reduction of the slow-wave frequency band far better than it did the alpha or beta bands (see Table 2-4). Scatterplots describing the change in slow-wave EEG power across age for the four 'EEG regions' can be seen in Figure 2-3.

EEG region	**β log (age)**	**t (β)**	**Sig (t)**	**R^2 change log(age)**	**R^2 model**	**F (model)**	**Sig (model)**
Slow-wave							
Frontal Lobe	-.5	-6.686	<.001	.237	.507	23.20	<.001
Temporal Lobe	-.57	-8.010	<.001	.312	.569	32.37	<.001
Parietal Lobe	.556	-7.750	<.001	.294	.316	31.02	<.001
Occipital Lobe	-.525	-7.122	<.001	.272	.273	25.40	<.001
Alpha							
Frontal Lobe	-.176	-2.059	.041	.03	.031	2.151	.120
Temporal Lobe	-.310	-3.765	<.001	.094	.095	7.092	.001
Parietal Lobe	-.258	-3.072	.003	.065	.066	4.725	.010
Occipital Lobe	-.275	-3.313	.001	.079	.082	6.043	.003
Beta							
Frontal Lobe	-.226	-2.739	.007	.042	.093	6.88	.001
Temporal Lobe	-.310	-3.838	<.001	.084	.126	9.76	<.001
Parietal Lobe	-.255	-3.136	.002	.064	.124	9.48	<.001
Occipital Lobe	-.361	-4.54	<.001	.116	.157	12.58	<.001

Table 2-4: Linear regression analyses for the absolute EEG power data, controlling for gender. Model: Regional Power = b0 + b1(gender) + b2(log(age)).

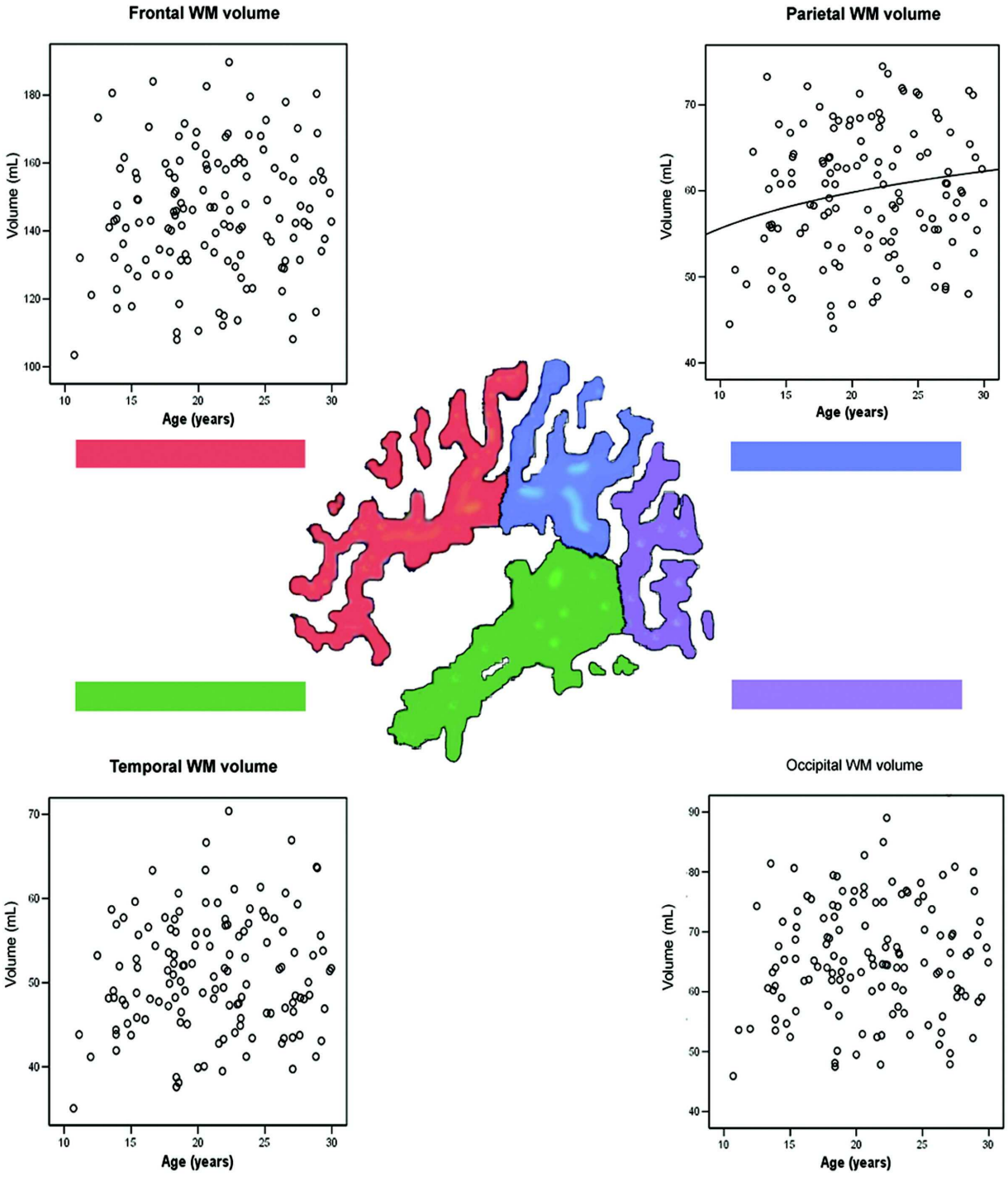

Figure 2-4: White matter vs age scatterplots for the frontal lobe, parietal lobe, occipital lobe and temporal lobe regions-of interest

The hypothesised relationship between the number of active synapses and absolute EEG power was supported by the significant positive correlations between subjects' GM volumes and their corresponding regional powers, particularly in the slow-wave frequency band (see Table 2-5) [2]. However, when slow-wave power and GM volume were both entered into the same regression analysis (controlling for gender), slow-wave power was found to be a substantially better predictor of log(age) than was GM volume for both the frontal (β = -.47, t = -5.87, p<.001 vs β = -.1, t = -1.13, p>.05) and parietal (β = -.54, t = -6.95, p<.001 vs β = -.06, t = -.71, p>.05) lobes. Thus the age-related changes in frontal and parietal GM volume and slow-wave EEG power, while mirroring each other in shape, were not completely parallel, due to the increased variability of the GM data.

MRI GM Supraregions	**Absolute power for the corresponding EEG regions**		
	Slow-Wave	**Alpha**	**Beta**
Frontal	.350 (p<.001)	.249 (p=.003)	.204 (p=.017)
Temporal	.214 (p=.013)	.235 (p=.006)	.102 (p=.236)
Parietal	.364 (p<.001)	.262 (p=.002)	.235 (p=.006)
Occipital	.345 (p<.001)	.295 (p<.001)	.274 (p=.001)

Table 2-5: Partial correlations (controlling for gender) between the volumes of the GM supraregions for the frontal, parietal, temporal and occipital lobes and the absolute power of the corresponding 'EEG regions' (e.g. frontal lobe GM with frontal lobe EEG power) for the slow-wave, alpha and beta frequency bands

White matter volume was found to increase linearly with the log of age only in the parietal WM ROI, although the log of age was close to being a statistically significant

[2] Similar results were observed for all analyses when the slow-wave band was separated into the delta (0.5-3Hz) and theta (3.5-7Hz) frequency bands

predictor of tissue volume in the remaining three WM ROIs (i.e. frontal, temporal and occipital), with the α level set at .01 (see Table 2-3 and Figure 2-4).

2.6 DISCUSSION

The primary purpose of this study was to investigate the structural and electrophysiological brain changes associated with healthy adolescence, and to examine the relationship between these changes. To this end, structural MRI and resting EEG data were recorded from 138 healthy participants aged between 10 and 30 years. After controlling for the effects of gender, grey matter volume was found to be negatively correlated with the log of age in the frontal and parietal cortices, while white matter volume was found to be positively correlated with the log of age in the parietal lobe. Absolute EEG power, which was averaged over the frontal, temporal, parietal and occipital cortices, was found to be negatively correlated with the log of age in all four 'EEG regions' for each of the three frequency bands, but most strongly in the slow-wave band. This age-related reduction in slow-wave power mirrored the age-related reduction in GM volume, especially for the frontal and parietal cortices (see Figure 2-3). This finding was supported by the observed positive correlation between subjects' frontal and parietal GM volumes and the absolute power of their corresponding 'EEG regions' (Table 2-5). These results provide evidence that changes in neural activity follow a similar trajectory to changes in brain structure over adolescence, at least in the frontal and parietal lobes.

The significant relationship between GM volume and the log of age in the frontal and parietal GM supraregions (see t(β) in Table 2-3) indicated that these regions experienced a period of significant tissue loss in adolescence, which decelerated in the second decade. However, the observed relationships between age and the volumes of the temporal, occipital or limbic GM supraregions were not statistically significant. This is consistent with previous studies that have found that while the association cortices undergo significant structural changes during adolescence and beyond (Thompson et al., 2001; Pfefferbaum et al., 1994; Steen et al., 1997), maturation of the evolutionary older limbic structures are largely complete by this time (Sowell et al., 1999), as are maturation of the primary sensory cortices (Huttenlocher, 1999), which constitute a large proportion of the temporal and occipital lobes. These results indicate that the time-course of grey matter maturation is heterogeneous across the brain.

Previous studies have indicated that rather than being due to neuron death, adolescent grey matter loss may rather be due to a programmed reduction in the number of synapses and their associated neuropil, i.e. dendrites, dendritic spines and axon terminals (Purves, 1998). There is evidence that a dramatic 'synaptic prune' occurs in healthy adolescence in the cortex of both humans (Huttenlocher et al., 1997) and primates (Rakic, Bourgeois, Eckenhoff, Zecevic, & Goldman-Rakic, 1986). Indeed, Bourgeois and Rakic (1993) used an electron micrograph to count the number of synapses in the visual cortex of macaque monkeys between the ages of 2.7 and 5 years (the period corresponding to adolescence) and estimated that they were losing up to five thousand synapses per minute over this period. In light of the fact that late adolescence and early adulthood is the most common

time for the onset of schizophrenia, a number of theorists have suggested that schizophrenia is caused by an abnormality in this 'synaptic prune' (Feinberg, 1982; McGlashan & Hoffman, 2000; Hoffman & Dobscha, 1989). Support for this theory comes from the fact that while patients with schizophrenia have a similar total number of neurons compared to controls (Feinberg, 1982; McGlashan et al., 2000; Pakkenberg, 1993), they show increased neuronal density, that is, an increased number of neurons per unit of cortical volume (Selemon & Goldman-Rakic, 1999). This finding is consistent with a reduction in cortical volume, possibly resulting from a reduction in the volume of neuropil in patients with schizophrenia (Selemon et al., 1999).

In contrast to the reductions in frontal and parietal GM volume, white matter volume was observed to *increase* curvilinearly (i.e. with the log of age) in only the parietal lobe, although the β-values for the frontal, temporal and occipital WM regions were close to significance at α=.01 (see Table 2-3 and Figure 2-4). This result further supports the idea that the association cortices undergo significant change in adolescence and early adulthood, with some studies finding that myelination of the association cortices is not complete until the late twenties (Yakovlev & Lecours, 1967). Previous research, however, has suggested that the rapid change that is present in adolescent GM development in not present in adolescent WM development, but rather that WM increases smoothly from birth, but at a decreasing rate with age (Pfefferbaum et al., 1994). An abnormality in adolescent myelination has also been proposed to be associated with the development of schizophrenia (Lim et al., 1998) . Given the role that myelin plays in modulating axonal conduction velocities, this hypothesis is especially salient in light of

theories that emphasise the role of neural timing in the development of disorganized thinking (Andreasen et al., 1999; Bartzokis, 2002).

Corresponding to the observed age-related reduction in grey matter volume, a similar curvilinear reduction in absolute power was also observed for each of the four 'EEG regions', across each of the three frequency bands (Table 2-4). This is consistent with the aforementioned hypothesis that a reduction in grey matter (irrespective of whether it reflected an elimination of neurons or neuropil) would result the elimination of synapses, which would lead to a reduction in amplitude of the EEG activity recorded at the scalp and thus a reduction in absolute EEG power. Although the curvilinear reduction in power with age was statistically significant for all three frequency bands, the relationship was far stronger - and mirrored the grey matter reduction better - in the slow-wave frequency band, as compared to the alpha or beta bands. It is possible to speculate as to why this might be:

The slow-wave frequency band is thought to arise primarily from highly synchronous local neural activity between cortical (pyramidal) neurons (Niedermeyer et al., 1999). This synchrony is responsible for the large amplitudes (and thus the large power) associated with slow-wave activity. Thus a large reduction in the number of synapses involved in slow-wave activity would be expected to result in a considerable loss of power. The beta frequency band, on the other hand, is thought to arise primarily from asynchronous activity between cortical pyramidal neurons, and is associated with low EEG power compared to the slow-wave band. This asynchrony implies that the

elimination of synapses involved in beta activity might actually result in an increase in power, if the pruned synapses were interfering destructively with others in the vicinity. It is thus possible that the relationship between synapse number and absolute power does not hold true for the high-frequency neural activity. Alternatively, the low amount of power associated with beta activity could result in any relationship with grey matter volume being indistinguishable from background variation (i.e. insufficient signal-to-noise). The alpha frequency (8-13Hz), on the other hand, is thought to be quite distinct from all other brain frequencies – the idiosyncratic alpha 'peak' on a typical EEG power spectrum being a testament to this. While the other frequencies are thought to arise from the synchronous and asynchronous 'chatter' between cortical neurons, alpha activity is thought to reflect highly synchronous cortical activity driven by the thalamus. Thus alpha activity might be expected to be influenced by structural changes in the thalamus, or thalamo-cortical relays, more so than changes in cortical grey matter *per se*. An alternative, and arguably more parsimonious, explanation lies in the fact that the occipital lobe (where alpha power is characteristically largest) did not show significant age-related changes in GM volume. Thus even if the hypothesised relationship between the number of synapses and EEG power held in the alpha frequency band, alpha would not be expected to show as predictable an age-related decrease in power in comparison to the slow-wave band, which has a more homogeneous spatial distribution (Niedermeyer et al., 1999).

Given the novelty of this research, these results (and the conclusions drawn from them) should be treated with caution until they are replicated. It would be useful to replicate this

study using a more exact non-linear spatial normalization procedure (e.g. Christensen, Rabbitt, & Miller, 1996) than the one applied here. It would be also be beneficial to replicate these results using a non-parametric MRI analysis technique (e.g. Bullmore et al., 1999), which make fewer assumptions of the data than the method described above. Incorporating the LORETA (Pascual-Marqui, Michel, & Lehmann, 1994) EEG source-localization technique into the analysis procedure would also be worthwhile, as it would provide a finer-grained approach to mapping the EEG signal to the underlying neuroanatomy than the method employed in this study.

In conclusion, this study found evidence of significant structural brain change in adolescence and early adulthood. Grey matter was observed to decrease at a decreasing rate in the frontal and parietal cortices, while white matter was observed to increase at a decreasing rate in the parietal lobe across the age range of 10 to 30 years. Curvilinear reductions in cortical EEG power, which mirrored the decreases in cortical grey matter, were also observed, especially in the slow-wave frequency band. I suggest that this reduction in EEG power is caused by a developmental period of 'synaptic pruning' that occurs in healthy adolescence.

CHAPTER 3

PROGRESSIVE GREY MATTER ATROPHY OVER THE FIRST 2-3 YEARS OF ILLNESS IN FIRST-EPISODE SCHIZOPHRENIA: A TENSOR BASED MORPHOMETRY STUDY

3.1 PREAMBLE

In many ways, the studies described in Chapters 3 and 4 form the cornerstone of this thesis. The primary aim of Chapter 3 was to investigate for evidence of grey matter abnormalities in patients with FES, both at the time of their first presentation to mental health services with psychotic symptoms (the 'baseline' study) and over the initial 2-3 years of illness (the 'follow-up' study). In the 'baseline study', the previously described technique of voxel-based morphometry was used to identify the regions of grey matter abnormality exhibited by 41 patients with FES, relative to 47 age and sex matched healthy controls. In the subsequent 'follow-up' study, the related technique of tensor-based morphometry was used to identify the regions in which a subset of 25 FES patients lost significantly more or significantly less grey matter volume over the 2-3 year follow-up interval, relative to a subset of 26 matched healthy controls.

By identifying, on a voxel-by-voxel basis, the regions of grey matter abnormality present in patients with FES, this study aimed at distinguishing between the validity of the various biological theories of schizophrenia described in Chapter 1. In particular, this study aimed to examine whether or not the first few years of illness was associated with a period of neurodegeneration in patients with first-episode schizophrenia.

The research described in this chapter has been published in the journal *NeuroImage* as an article entitled: "Progressive grey matter atrophy over the first 2-3 years of illness in

first episode schizophrenia: a tensor-based morphometry study" (see Whitford et al., 2006 and Appendix 3).

3.2 ABSTRACT

Little is known about the structural brain changes that occur over the first few years of illness in schizophrenia, or how these changes differ from those associated with healthy brain development in adolescence and early adulthood. The aim of this study was to identify the regional differences in grey matter (GM) volume between patients with first-episode schizophrenia (FES) and matched healthy controls, both at the time of patients' first presentation to mental health services with psychotic symptoms (baseline condition) and 2-3 years subsequently (follow-up condition). Forty-one patients with FES and 47 matched healthy controls underwent a T1-weighted structural MRI scan. Of these participants, 25 FES patients and 26 controls returned 2-3 years later for a follow-up scan. Voxel-based morphometry in SPM2 was used to identify the regions of GM difference between the groups in the baseline condition, while tensor-based morphometry was used to identify the longitudinal change within-subjects over the follow-up interval. The FES patients exhibited widespread GM reductions in the frontal, parietal and temporal cortices and cerebellum at baseline, as well as more circumscribed regions of GM increase, particularly in the occipital lobe. Furthermore, the FES subjects were observed to lose considerably more GM over the follow-up interval than the controls, especially in the parietal and temporal cortices. I propose that the progressive GM

atrophy observed in this study arises from a dysfunction in the dramatic period of healthy brain development typically associated with adolescence.

3.3 INTRODUCTION

It has been well established that patients experiencing their first episode of schizophrenia (FES) exhibit structural brain abnormalities relative to matched healthy controls. Previous studies using structural magnetic resonance imaging (MRI) have consistently found evidence of grey matter (GM) atrophy in FES patients, especially in the frontal (Job et al., 2002; Salokangas et al., 2002), temporal (Kubicki et al., 2002; Park et al., 2004) and parietal cortices (Narr et al., 2005; Nierenberg et al., 2005) and in the limbic system (Joyal et al., 2002; Bogerts et al., 1990). A dysfunction in the intense period of targeted synaptic elimination that is associated with healthy adolescence has been proposed as the mechanism underlying this pathological GM loss (Feinberg, 1982; Keshavan et al., 1994). The results of these studies are particularly informative, given as they are free from the confounds associated with chronic exposure to neuroleptic medication, which may well influence brain structure in and of itself (Madsen et al., 1999; Lieberman et al., 2005).

This period of GM atrophy may be localised in time or, alternatively, may be the harbinger of a progressive ongoing pattern of GM loss that continues throughout adulthood. The temporal nature of the course of GM loss associated with FES, besides being of fundamental importance in understanding the nature of the disease, has

important implications for treatment, as if cerebral atrophy is degenerative then arresting this degeneration might prevent the debilitating cognitive and social decline associated with chronic schizophrenia (Milev, Ho, Arndt, & Andreasen, 2005). Although a number of studies have investigated this question via a cross-sectional design in which subjects of different ages are each scanned once and age-related trends in brain volume are inferred with a regression model (Hulshoff Pol et al., 2002; Steen et al., 1997), a longitudinal design in which each subject is scanned several times over a period of years, is more statistically powerful, due to a reduction in the within-subjects variance. Despite this theoretical advantage, however, the results of previous longitudinal studies in FES have been somewhat equivocal. Whilst several have reported evidence of progressive grey matter atrophy in FES patients, for both whole-brain volume (Cahn et al., 2002b) and regional volumes including frontal lobe (Mathalon, Sullivan, Lim, & Pfefferbaum, 2001), temporal cortex (Kasai et al., 2003) and hippocampus (Lieberman et al., 2001), others have failed to find evidence of progressive cerebral degeneration (DeLisi et al., 1992; Whitworth et al., 2005; DeLisi & Hoff, 2005).

A possible reason for the inconsistent results is that different studies often examine different regions of interest (ROIs) for evidence of structural brain abnormality. This is one area in which the automated statistical imaging techniques (e.g. voxel based morphometry) are particularly advantageous – they look for changes at every voxel in the brain (given appropriate statistical correction), and are therefore not constrained to the regions defined by prior hypotheses. Thompson et al. (2001) used a statistical imaging technique to investigate evidence of progressive structural brain changes in

schizophrenia. They demonstrated that initial GM reductions in the parietal lobes of childhood-onset patients progressed anteriorly over the next five years, and engulfed the prefrontal and temporal cortices, areas which have regularly been found to be atrophied in patients with chronic schizophrenia (McCarley et al., 1999).

Voxel-based morphometry (VBM) has been used previously to identify differences in GM atrophy over 2-3 years between FES patients and first-episode bipolar patients (see Appendix 2). In this study, VBM was used to identify the differences in regional GM volume between 41 patients with first-episode schizophrenia and 47 matched healthy controls, within three months of the patients' first presentation to mental health services with psychotic symptoms (baseline condition). Tensor-based morphometry (TBM) was then used to investigate the change over the next 2-3 years in regional GM volume in 25 of the FES patients relative to 26 of the matched healthy controls (follow-up condition). In TBM, each subjects' baseline image is warped directly to their follow-up image prior to normalization to a T1-template, which results in an increased sensitivity (relative to VBM) in detecting structural brain changes over time.

Based on previous research, it was hypothesised that the patients with schizophrenia would show evidence of widespread cortical atrophy at the time of their first psychotic episode (the baseline condition), especially in the frontal and temporal cortices. Furthermore, it was hypothesised that the FES patients would lose GM at a faster rate than matched healthy controls over the subsequent 2-3 years (the follow-up condition), largely in the same regions in which they showed atrophy at baseline.

3.4 METHODS

3.4.1 Participants

Forty-one patients experiencing their first episode of schizophrenia were recruited for the baseline condition as part of the Western Sydney First Episode Psychosis project, a multimodal project investigating the clinical, neuroanatomical, neuropsychological and psychophysiological profiles of young people in western Sydney experiencing their first-episode of psychosis (Harris et al., 2005). A stringent criterion for first episode status was employed whereby all patients were recruited within 3 months of their first presentation to mental health services with psychotic symptoms (defined as hallucinations, delusions, formal thought disorder or prominent negative symptoms that persisted for a minimum of 3 days), although some patients had previously presented with symptoms of anxiety and depression that were not judged to be psychotic at the time. Diagnosis of schizophrenia was made using DSM-IV criteria (American Psychiatric Association, 1994), by a consensus conference of at least three senior psychiatrists, at least two of whom were independent of the study. Subjects with schizophrenia were interviewed and rated by psychiatrists who had reached an acceptable level of inter-rater reliability ($r > .8$) on the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1989).

Forty-seven healthy control subjects were recruited for comparison from parallel geographical regions in collaboration with the Brain Resource International database [http://www.brainresource.com (Gordon, 2003)]. Control subjects were screened for the

presence of an Axis-I disorder, using the SPHERE (Hickie et al., 1998), and subjects were also excluded if they reported a first-degree family member with an Axis I diagnosis. Controls were within the normal range on depression, anxiety and stress, assessed using an abbreviated version of the Depression Anxiety and Stress Scale (DASS) (Lovibond & Lovibond, 1995).

Exclusion criteria for both groups were a past history of substance dependence, exposure to electroconvulsive therapy within the past 6 months, mental retardation (estimated pre-morbid IQ <75, based on Wide Range Achievement Task Revision 3 (WRAT-3) (Wilkinson, 1993) and the Spot-the-Real-Word test (Baddeley, Emslie, & Nimmo-Smith, 1993)), neurological disorder including epilepsy, and a history of head injury causing loss of consciousness for at least 1 hour. After a detailed description of the study, each subject gave written informed consent to participate, in accordance with Australian National Health and Medical Research Council guidelines. There were no significant differences between the FES and control subjects with respect to age-at-scan, gender, handedness or estimated pre-morbid IQ at baseline. Of the 41 FES subjects and 47 matched healthy controls scanned in the baseline condition, 25 FES and 26 control subjects returned between two and three years later (on average) for a follow-up scan. There were no significant differences between the FES and control subjects with respect to age-at-scan, gender, handedness, estimated pre-morbid IQ or time interval between scans (follow-up interval). Furthermore, there were no significant differences observed between the baseline and follow-up samples with respect to age, gender handedness and IQ for the control subjects, and age, gender, handedness, IQ, duration of untreated psychosis and

scores on the PANSS Positive, Negative and General rating scales for the FES subjects. This suggested that the follow-up sample was representative of the baseline sample for both the FES and control subjects. The demographic data for the patients and controls at baseline and follow-up is presented in Table 3-1. Over the course of the study, patients received treatment from a variety of services and no fixed treatment protocol was used. Treatment combined second-generation antipsychotics (amisulpride, clozapine, olanzapine, risperidone and quetiapine) with community case management, which varied from once or twice weekly contact with psychosocial rehabilitation to occasional contact from subjects refusing services. Most patients were exposed to at least two antipsychotics over the course of the follow-up interval. This study was approved by the Western Sydney Area Health Service Human Research Ethics Committee.

Baseline	FES (n = 41)	Healthy Controls (n=47)
Age-at-scan (years)	19.8 (3.3) [13 – 25]	19.3 (3.8) [12 – 28]
Gender	26M, 16F	33M, 14F
Handedness	33R, 7L	39R, 8L
DUP (months)	8.3 (10.6) [0 – 38]	-
Medication dosage (CPZ)	236 (200) [0 – 667]	-
PANSS Positive	17.7 (5.7) [7 –29]	-
PANSS Negative	18.8 (6.4) [7 – 35]	-
PANSS General	40.3 (8.4) [23 – 58]	-
Pre-morbid IQ estimate	100 (9.5) [77 – 124]	104 (8.2) [80 – 117]
Follow-Up	**FES (n=25)**	**Healthy Controls (n=26)**
Follow-up interval (months)	30.9 (6) [23 – 40]	28.2 (6.6) [24 – 53]
At-at-scan (years)	22.1 (3.2) [15-27]	22 (4.4) [14-30]
Gender	15M, 10F	15M, 11F
Handedness	21R, 4L	20R, 6L
DUP (months)	5.9 (8.2) [0-36]	-
Medication dosage (CPZ)	280 (272) [0 – 833]	-
PANSS Positive	14.6 (6.1) [7 –32]	-
PANSS Negative	15 (6.5) [7 –30]	-
PANSS General	30.5 (9.1) [17 – 50]	-
Pre-morbid IQ estimate	100 (11.1) [77-124]	105 (9.2) [80-117]

Table 3-1: Demographic details of the subject sample for both the baseline and follow-up studies, with the mean, standard deviation and range provided

3.4.2 MRI Acquisition: baseline study

Both FES and control subjects underwent a single T1 weighted volumetric MPRAGE structural MRI scan on a Siemens 1.5-Tesla Vision Plus system at Westmead Hospital, Sydney. All images for the baseline study were obtained coronally with the following parameters: TR=9.7 ms, TE=4 ms, TI=200 ms, flip angle=12º, FOV=256mm, matrix 192x256, voxel size=1mm^3. The control subjects were also scanned sagittaly at baseline

on the same MR scanner, and this sagittal scan was used to compare to the controls' sagittal follow-up scan, as discussed below.

3.4.3 MRI Acquisition: longitudinal study

The scanning protocol for the follow-up FES subjects was identical to that described for the baseline study (i.e. a coronally-acquired MPRAGE scan). The control subjects, however, underwent a sagittal MPRAGE scan, which was identical to the sagittal scan they received at baseline, as mentioned above. The following parameters were used for the sagittal scan: TR=9.7 ms, TE=4 ms, TI=200 ms, flip angle=12º, FOV=256mm, matrix 256x256, voxel size=1mm^3. All imaging was performed on the same MR scanner. There were no significant upgrades or modifications of the scanner between the baseline and follow-up stages of the study. To control for scanner drift, phantom data was collected weekly over the follow-up interval, and the scanner calibrated accordingly.

3.4.4 Image Pre-Processing: baseline study

The baseline images were processed using voxel-based morphometry (VBM) in SPM2 (Wellcome Department of Cognitive Neurology, London, UK), running on Matlab 6.5 (MathWorks, Natick, USA). The full details of the processing protocol used in VBM are presented elsewhere (Ashburner et al., 2000; Good et al., 2001). Briefly, subjects' brain images were first spatially normalized to a customised template, which was created by averaging the smoothed (with an 8mm full-width at half-maximum Gaussian kernel),

normalised whole-brain images from all subjects that had been registered to the ICBM 152 template (Montreal Neurological Institute) that approximates Talairach space. The first step in spatial normalization involved estimating the optimum 12-parameter affine transformation (3 translations, 3 rotations, 3 zooms and 3 shears) for matching the subject's image to the template. The second step accounted for global non-linear shape differences, which were modelled by a linear combination (7x8x7) of smooth spatial basis functions (Ashburner et al., 2000). The normalized images were re-sliced with $1.5mm^3$ voxels, before being segmented into GM, white matter (WM) and cerebrospinal fluid (CSF) probability maps, and stripped of extra-cerebral voxels. Segmentation was based on a cluster analysis method that accounted for each voxel's signal intensity, together with an *a priori* expectation of the anatomical location of the different tissue types. In order to adjust for the growth and shrinkage of voxels that can occur during spatial normalisation, voxel probability values in the cleaned, segmented images were modulated with the Jacobian determinants derived from the spatial normalization (Good et al., 2001). Thus if a brain region doubled in size as a result of normalization, the grey matter probability value for this region would be halved for the purposes of calculating its volume (Ashburner et al., 2001). The processed GM, WM and CSF images were smoothed with a 12mm Gaussian kernel, prior to volume calculation.

3.4.5 Image Pre-processing: longitudinal study

The methodology used for tensor-based morphometry followed the methodology described by Kipps et al. (2005). The following procedure was applied to the scans of each subject in SPM2:

1. A rigid body transformation (3 translations and 3 rotations) was initially used to approximately register the baseline to the follow-up (FU) image. The quality of the registration was manually checked and was deemed acceptable for all images. All images were re-sliced into $1.5mm^3$ voxels.

2. Using the 'spm_warp_ui.m' script (written by John Ashburner; http://www.fil.ion.ucl.ac.uk/~john) in the 'Deformation Toolbox' in SPM2, a high-dimensional deformation field was sought that would warp each subjects' baseline image to their FU image as closely as possible (Ashburner, Andersson, & Friston, 2000), by minimising the mean squared difference between the images. The regularisation parameter, which defined the compromise between the mean squared difference between the images and the smoothness of the deformations, was set to four. Eight iterations of the deformation algorithm were performed. The result of the high-dimensional warp was a deformation field that contained the information required to map a point on the FU image to a corresponding point on the baseline image, for each subject. The amount of regional expansion or contraction was extracted from this deformation field, by taking the Jacobian determinant (i.e. the determinant of the gradient of the deformation) at each

point (Freeborough et al., 1998). An image consisting of the Jacobian determinants at each point was generated in alignment with the FU image. This Jacobian 'map' encoded the number of cubic millimetres in the baseline image that corresponded to one cubic millimetre in the FU image. For example, if a brain structure uniformly contracted from $20mm^3$ to $10mm^3$ in a particular subject over the follow-up interval, then the value of the Jacobian determinant would be 2 in voxels in the Jacobian map corresponding to that structure. The baseline images were not used again in subsequent pre-processing or analysis.

3. The FU images were normalised to a customised whole-brain template, which was generated by averaging the smoothed (with an 8mm Gaussian kernel), normalised whole-brain images of all the FU images, which had been registered to the ICBM 152 template. The normalised FU images were then segmented into GM, WM and CSF images, and extra-cerebral voxels removed. The procedure for normalisation and segmentation was identical to that as described for the baseline study. Each subjects' spatial normalisation parameters were then applied to their Jacobian map generated in step 2.

4. Each subjects' normalised GM segment (from step 3) was multiplied, voxel-by-voxel, with their normalised Jacobian map (from step 3) to form a product image. As regional contraction over the follow-up interval corresponded to a Jacobian determinant > 1 (see step 2), voxel values in the product image exceeded corresponding values in the late tissue segment in regions where tissue contraction had occurred.

5. Each subjects' GM segment and product image from step 4 was modulated with the Jacobian determinants from the spatial normalization described in step 3. The modulated images were then smoothed with a 12mm Gaussian kernel. These smoothed product and GM segment images were entered into the statistical analysis.

3.4.6 Statistical Analysis: baseline study

Statistical analyses (which can be regarded as ANCOVAs (Friston et al., 1995)) were undertaken in SPM2 to identify the brain regions where the FES patients exhibited GM volume reductions relative to the healthy controls. Subjects' age-at-scan, gender, handedness and global GM volume (calculated by summing the voxel values in each subject's pre-processed GM image) were included as nuisance covariates in the analysis. Output was in the form of Statistical Parametric Maps (SPMs), based on a voxel-level height threshold of $P < 0.05$ (corrected for multiple comparisons using Gaussian random field theory; (Worsley et al., 1996)) and a cluster-level extent threshold of 100 contiguous voxels. Coordinates for foci of maximal GM change within each supra-threshold cluster were produced as MNI coordinates. To facilitate interpretation of results relative to previous studies, these MNI coordinates were transformed into Talairach (Talairach et al., 1988) coordinates using the 'mni2tal.m' Matlab script written by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging/Common).

3.4.7 Statistical Analysis: longitudinal study

The general linear model in SPM2 was used for statistical inference. The images generated from step 5 of the longitudinal-study image pre-processing protocol were entered into the design matrix in four conditions: control product images, control GM segments, schizophrenia product images and schizophrenia GM segments. Fifty-one subject-specific, dummy covariates (i.e. 25 schizophrenia and 26 control subjects) modelled the variance attributable to repeated measures within subject. The interaction between subjects' diagnosis (i.e. control or schizophrenia) and image type (i.e. product or GM segment) was examined with a [-1 1 1 -1] contrast of parameter estimates for each voxel, using 2-tailed *t*-statistics. In other words, SPM performed a paired *t*-test to determine whether greater tissue contraction had occurred in the patients with schizophrenia relative to the control subjects. Subjects' age, gender, handedness and follow-up interval were entered as nuisance covariates in the analysis. Output was again in the form of Statistical Parametric Maps (SPMs), based on a voxel-level height threshold of $P < 0.05$ (corrected for multiple comparisons) and an extent threshold of 100 contiguous voxels. As for the analysis of the baseline condition, the 'mni2tal.m' Matlab script was used to transform the MNI coordinates into Talairach coordinates.

3.5 RESULTS

3.5.1 Baseline study

The 41 FES patients showed widespread GM reduction relative to the 47 matched healthy controls at baseline, after controlling for subjects' age, gender, handedness and global GM volume (Figure 3-1 and Table 3-2). The right frontal and parietal cortices and the left parietal and temporal cortices were the regions most reduced in the FES patients, although there were also significant reductions in the left ventral prefrontal cortex and right inferior temporal cortex and cerebellum.

Anatomical Region (local maxima >8mm apart per cluster)	**T&T co-ordinate of voxel of maximum significance**			**T-value**	**Cluster size**
	x	y	z		
Precentral Gyrus	64	-2	27	8.17	3819
Inferior Frontal Gyrus	61	7	26	8.05	
Precentral Gyrus	60	-11	42	7.22	
Lingual Gyrus	30	-60	-4	7.48	712
Middle Frontal Gyrus	-32	51	-7	7.45	816
Middle Frontal Gyrus	-27	52	8	6.76	
Middle Frontal Gyrus	-38	43	-6	6.38	
Middle Frontal Gyrus	-62	21	37	7.34	2859
Precentral Gyrus	-60	-15	41	7.14	
Middle Temporal Gyrus	-68	-32	-3	6.91	
Inferior Frontal Gyrus	-17	25	-14	6.22	288
Inferior Frontal Gyrus	-26	28	-13	5.77	
Anterior Cingulate Gyrus	-11	36	17	5.77	186
Fusiform Gyrus	39	-42	-27	5.2	220

Table 3-2: Baseline Study – regions where the 41 FES patients had less GM at baseline than did the 47 matched healthy controls (statistically controlling for age, gender, handedness and global GM volume)

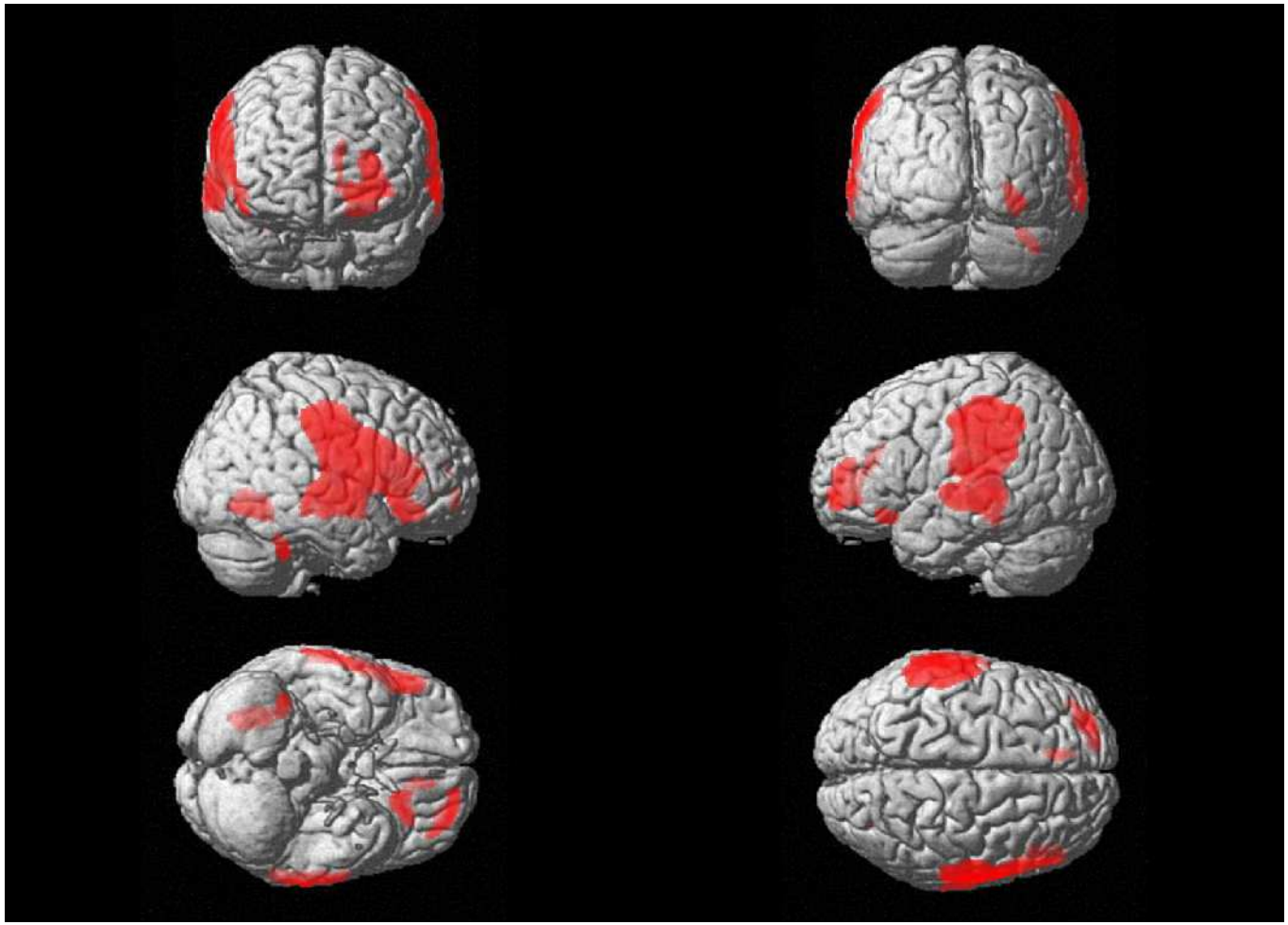

Figure 3-1: Regions of reduced grey matter volume at baseline in 41 FES patients compared to 47 matched healthy controls. The regions of reduction are displayed as a rendered, three-dimensional statistical parametric map (SPM); height threshold: $p<.05$ corrected for family-wise error, extent threshold = 100 voxels.

There were also a number of regions where the FES patients showed increased GM relative to the matched healthy controls at baseline (Figure 3-2 and Table 3-3). There was a large region of increase in the occipital lobe, and smaller regions in the superior and middle frontal gyri, precentral gyri, claustrum and cerebellum.

Anatomical Region (local maxima >8mm apart per cluster)	**T&T co-ordinates of voxel of maximum significance**			**T-value**	**Cluster size**
	x	y	z		
Lingual Gyrus	21	-96	-3	9.9	3387
Cuneus	-15	-99	5	9.23	
Inferior Occipital Gyrus	27	-94	-7	8.83	
Superior Frontal Gyrus	-12	56	32	6.87	222
Superior Frontal Gyrus	-8	39	48	5.95	
Superior Frontal Gyrus	-9	51	38	5.37	
Superior Frontal Gyrus	-39	45	25	6.49	138
Middle Frontal Gyrus	-41	50	14	5.85	
Superior Frontal Gyrus	15	56	32	6.27	208
Superior Frontal Gyrus	9	51	39	5.93	
Superior Frontal Gyrus	9	60	25	5.45	
Precentral Gyrus	18	-16	62	6.25	612
Claustrum	39	-18	-4	5.55	178
Precentral Gyrus	-14	-16	59	5.49	106
Cerebellum	-15	-89	-41	5.27	204

Table 3-3: Baseline study - regions where the 47 matched healthy controls had less GM at baseline than did the 41 FES patients (statistically controlling for age, gender, handedness and global GM volume)

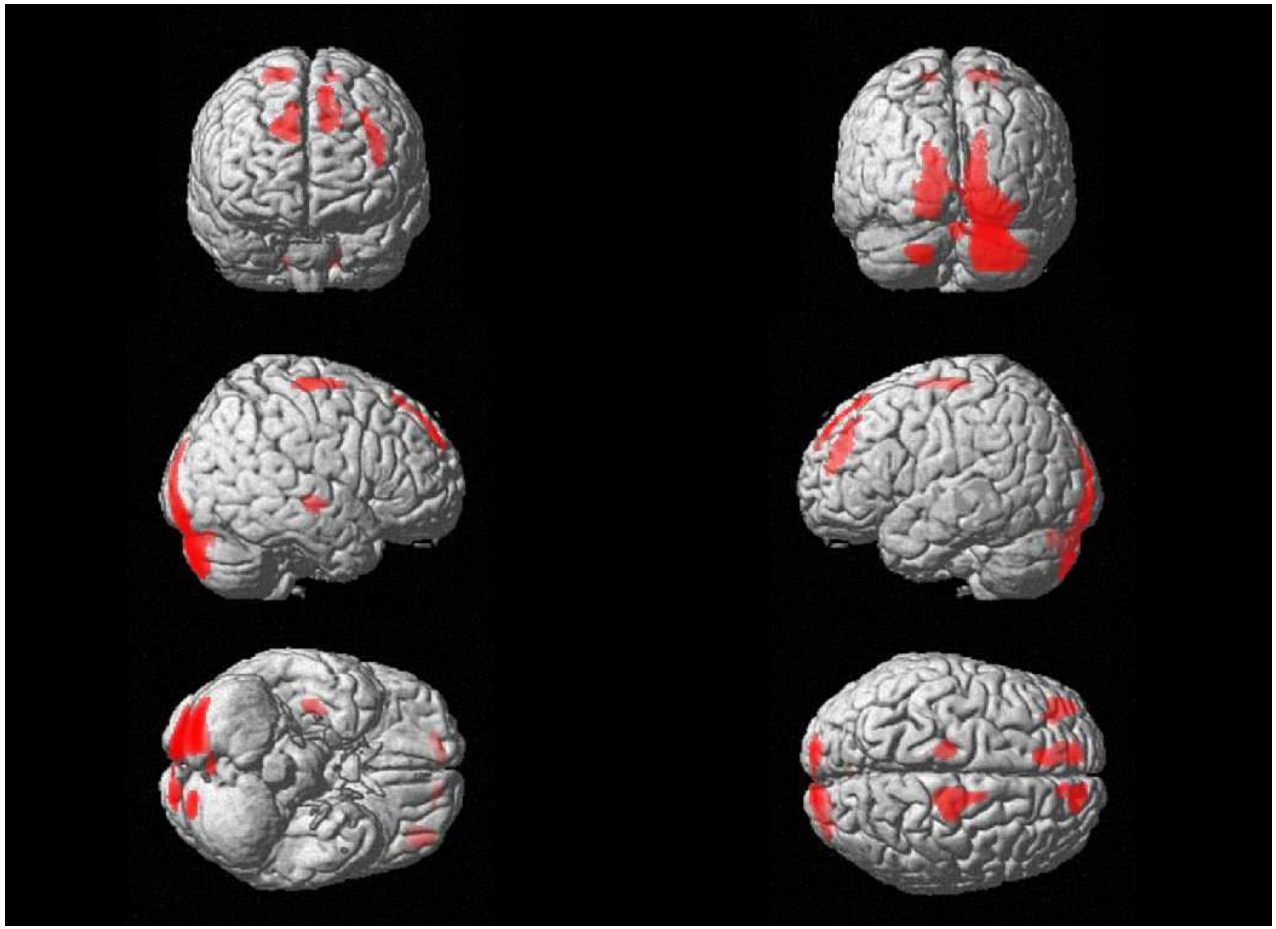

Figure 3-2: Regions of increased grey matter volume at baseline in 41 FES patients compared to 47 matched healthy controls. The regions of increase are displayed as a rendered, three-dimensional statistical parametric map (SPM); height threshold: $p<.05$ corrected for family-wise error, extent threshold = 100 voxels.

3.5.2 Longitudinal study

The 25 FES subjects showed extensive GM loss over the 2-3 year follow-up interval over and above the GM loss experienced by the 26 matched healthy controls (Figure 3-3 and Table 3-4). The parietal and temporal lobes bilaterally were most affected, although atrophy extended into the posterior ventral prefrontal cortex and cerebellum. There were no regions in which the 26 healthy controls were found to lose more GM over the follow-up interval than the 25 FES patients.

Anatomical Region (local maxima >8mm apart per cluster)	**T&T co-ordinates of voxel of maximum significance**			**T-value**	**Cluster size**
	x	y	z		
Precuneus	10	-70	43	8.59	11264
Sub-gyral Temporal Lobe	-49	-17	-21	8.57	
Precuneus	-4	-72	46	8.53	
Superior Temporal Gyrus	34	16	-24	7.5	824
Superior Temporal Gyrus	38	6	-26	7.04	
Sub-gyral Temporal Lobe	42	-11	-21	6.77	
Parahippocampal Gyrus	16	-46	8	7.15	319
Lingual Gyrus	18	-41	0	7.07	
Posterior Cingulate Gyrus	8	-50	14	6.29	
Cerebellum	6	-52	-39	6.79	190
Cerebellum	10	-54	-33	6.4	
Cerebellum	-4	-54	-39	6.29	
Cerebellum	-22	-77	-25	6.71	574
Cerebellum	-24	-75	-33	6.34	
Cerebellum	-16	-74	-38	5.62	
Inferior Frontal Gyrus	-32	24	-18	6.62	130
Inferior Frontal Gyrus	-40	26	-18	5.96	
Inferior Frontal Gyrus	-30	18	-23	5.58	
Postcentral Gyrus	57	-16	21	6.45	664
Inferior Parietal Lobule	53	-29	33	6.24	
Superior Temporal Gyrus	61	-27	9	6.17	

Table 3-4: Longitudinal study – regions where the 25 FES patients lost more GM over the FU interval than did the 26 matched healthy controls (statistically controlling for age, gender, handedness and FU interval)

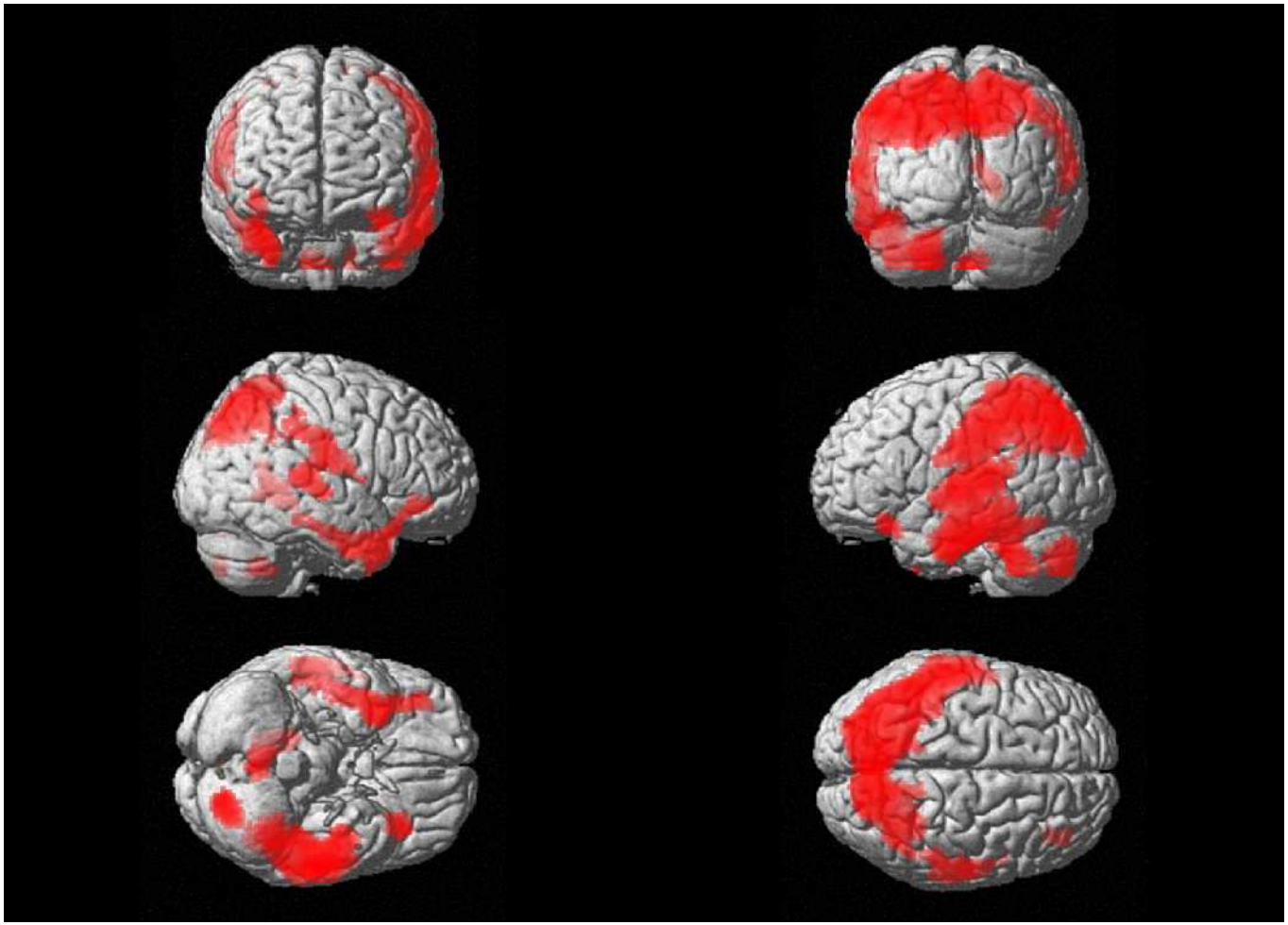

Figure 3-3: Regions where 25 FES patients lost more grey matter volume over the 2-3 year follow-up interval compared to 26 matched healthy controls. These regions are displayed as a rendered, three-dimensional statistical parametric map (SPM); height threshold: $p<.05$ corrected for family-wise error, extent threshold = 100 voxels. There were no regions in which the 26 controls were observed to lose more grey matter over the follow-up interval compared to the 25 FES patients, given the height and extent threshold.

3.6 DISCUSSION

In this study, voxel-based morphometry (VBM) was used to identify GM abnormalities across the brain in 41 patients with first-episode schizophrenia (FES) relative to 47 matched healthy controls in a baseline study performed when patients were recruited. Substantial regional GM abnormalities were observed in the FES subjects at baseline, including reductions in the parietal, temporal and ventral frontal cortices and cerebellum, and increases in the occipital lobe, dorsal frontal cortex and primary motor cortex (Tables 3-2, 3-3 and Figures 3-1, 3-2). Tensor-based morphometry was used to quantify the progressive GM change over the next 2-3 years in a subset of the baseline subjects (25 FES and 26 healthy controls). The FES subjects were observed to lose substantially more GM than the controls over the follow-up interval, especially in the parietal cortices and posterior temporal cortex. There were no regions in which the control subjects lost more GM in the follow-up interval than did the FES subjects (Table 3-4 and Figure 3-3). In short, these results suggest that patients with schizophrenia already exhibit GM abnormalities relative to controls at the time of their first psychotic episode, and that GM loss continues in patients with schizophrenia over the next 2-3 years, at a greater rate than that experienced by healthy subjects.

The results of the baseline study are generally consistent with the results of previous studies into FES, with reductions in the frontal, temporal and parietal cortices commonly reported (Job et al., 2002; Kubicki et al., 2002; Narr et al., 2005). Reports of reductions in the cerebellum are also not unprecedented in the literature (Ichimiya, Okubo, Suhara, &

Suda, 2001), and have been used to support the theory that rather than being exclusively responsible for motor coordination, the cerebellum plays a role in coordinating the timing of the higher-level cognitive activity that is typically dysfunctional in schizophrenia (Andreasen et al., 1999). Based on the results of this study and previous research, there is very strong evidence to suggest that patients with schizophrenia exhibit GM deficits at the time of their first psychotic episode. There is even evidence to suggest that structural GM abnormalities may in fact precede the onset of psychotic symptoms in high-risk subjects who go on to develop schizophrenia (Pantelis et al., 2003). The question of whether the onset of psychosis can be prevented following the development of these structural brain abnormalities has not been well addressed in the literature, and remains a research area of enormous clinical significance.

There were similarities but also notable differences in the regions where the FES patients exhibited GM reductions at baseline compared to where they exhibited increased progressive GM atrophy over the follow-up interval, compared to the healthy controls. For example, while widespread reductions in the prefrontal cortex were observed in the FES patients at baseline, the FES patients showed comparatively little progressive GM loss in this region compared to the healthy controls. The parietal lobe, on the other hand, was heavily reduced at baseline in the FES patients, and was also the site of accelerated GM loss over the follow-up period in the patients relative to the healthy controls. This finding is consistent with the results of a previous longitudinal study by our group, which employed a different methodology to compare the longitudinal GM atrophy in FES and first-episode bipolar patients (see Appendix 2). It is not entirely consistent, however, with

the results of a recent meta-analysis by Honea et al. (2005) who reported frontal lobe GM atrophy as being among the most consistently reported findings in previous VBM studies into schizophrenia. A notable feature of the Honea et al. (2005) study, however, was that the majority of the studies it included had investigated patients with chronic schizophrenia, as opposed to the first-episode sample described here. Thus it is possible that the widespread parietal lobe atrophy observed in the FES patients in this study would progress to the frontal lobe as the disease became chronic. This proposal is consistent with the findings of Thompson et al. (2001), who observed a dynamic wave of progressive GM atrophy in patients with early-onset schizophrenia, which began in the parietal cortices and progressed anteriorly over the subsequent five years to the temporal and finally the frontal cortices. Hence it appears as though progressive abnormalities of the parietal cortices may be characteristic of the early stages of schizophrenia. The role of the parietal cortices in the development of psychotic symptoms in schizophrenia has been established previously, particularly in regards to the development of symptoms of reality distortion (Shergill, Brammer, Williams, Murray, & McGuire, 2000; Spence et al., 1997; see also Chapter 5), with an emphasis being placed on its role in the monitoring of internally generated thoughts and actions. Furthermore, abnormalities in these processes of self-monitoring have previously been proposed as the neural bases for hallucinations and delusions (Frith, 2005). Identifying the mechanisms underlying the abnormal progressive GM atrophy in the early stages of schizophrenia is essential if this atrophy is to be successfully arrested.

Progressive GM loss in the years immediately following psychosis onset in adult-onset schizophrenia have been reported by the majority of the few studies previously undertaken (Lieberman et al., 2001; Ho et al., 2003; Cahn et al., 2002b), although there have been exceptions (DeLisi et al., 1992). In contrast, few of the previous longitudinal studies have identified evidence of progressive GM loss in patients with chronic schizophrenia (Lauriello et al., 1997; Vita, Dieci, Giobbio, Tenconi, & Invernizzi, 1997), although again there have been conflicting findings (Mathalon et al., 2001). Gur et al. (1998a) directly compared the longitudinal changes in frontal and temporal lobe volume over 2-4 years in first-episode and chronic schizophrenic patients, and healthy controls and found that the first-episode patients lost significantly more GM than did chronic patients over the follow-up interval, while the chronic patients did not, in general, lose any more GM than controls. The study described in this chapter provides evidence of diffuse, progressive GM loss occurring in patients with FES patients over only a 2-3 year period. Thus it seems likely that there is a period of progressive GM loss in the years immediately following the onset of symptoms in schizophrenia, and that this rate of loss attenuates later in the course of the disease. It is possible to speculate as to why this might be:

The most common age-of-onset for schizophrenia is late adolescence and early adulthood, which is consistent with the average age of the FES sample in this study, which was 19.6 years for the males and 20 years for the females. This age corresponds to a period of enormous structural change in the healthy human brain (Purves, 1998; Sisk et al., 2004), with the elimination of millions of synapses and their associated neuronal

processes (dendrites and axon terminals). This ‘synaptic prune’ (which has been associated with the development of the sustained logical thought in adulthood; (Feinberg, 1982)) has been suggested to arise from a reduction in neuroprotective trophic factors, possibly in response to puberty-related hormonal changes (Purves, 1998). It has been suggested that schizophrenia arises from a dysfunction in this period of healthy brain maturation (Feinberg, 1982; Keshavan et al., 1994), possibly due to abnormalities in the genes coding for these trophic factors (e.g. see Hakak et al., 2001). This could explain the general finding of progressive structural brain atrophy early in the course of schizophrenia, but not in the later stages of the disease. In this case it would be conceivable that an abnormality in the mechanism responsible for this ‘synaptic prune’ could result in the survival of synapses that would otherwise have been pruned, as the well as the elimination of synapses that would otherwise have been preserved. Such a scenario has previously been proposed to underlie the disorganization of thought typically associated with schizophrenia (Chua et al., 1997), and might account for the observation of regional GM increase in the FES patients at baseline, particularly in the occipital lobe and cerebellum.

In summary, this study found evidence that patients with schizophrenia exhibit widespread GM deficits at the time of their first psychotic episode relative to matched healthy controls, and that they lose more GM in the subsequent 2-3 years than do controls, especially in the parietal cortices. I have suggested that these progressive volumetric GM abnormalities arise from a dysfunction in the period of neural reorganization associated with adolescence and early adulthood (the most common age of

onset in schizophrenia), and subsequently might be confined to this period, rather than degenerating further with illness chronicity. By rescanning these same participants at regular intervals over the next decade, I am hoping to be able to test this hypothesis, as any structural brain changes due to peripubertal neurodevelopment would be expected to be complete by the end of this period. There is an enormous cost, both socially and economically, associated with schizophrenia given that it is a life-long chronic, disabling and progressive condition. Thus even small changes in disease severity that might be achieved by investigating and targeting the peripubescent onset of the illness would bring enormous health and cost benefits - arguably as big as any in the health care world.

CHAPTER 4

VOLUMETRIC WHITE MATTER ABNORMALITIES IN FIRST-EPISODE SCHIZOPHRENIA: A LONGITUDINAL MRI STUDY

4.1 PREAMBLE

The research described in Chapter 3 identified grey matter abnormalities in patients with FES both at the time of their first presentation to mental health services with psychotic symptoms (baseline) and over the subsequent 2-3 years of illness (follow-up). The research described in this chapter used the same methodology and investigated the same subject sample as the research described in Chapter 3. The only difference was that while the study described in Chapter 3 investigated for *grey matter* abnormalities in the FES patients, the study described in this chapter investigated for *white matter* abnormalities in these patients, both at baseline and over the follow-up interval.

As discussed in Chapter 1, a number of prominent theories of schizophrenia (e.g. Andreasen, 1999; Bartzokis, 2002; Frith, 1992) have proposed a dysfunction in neural connectivity as being the primary cause of the disease. Bartzokis (2002), in particular, emphasized the role of dysfunctional neural timing resulting from abnormal myelination in the formation of the symptoms of schizophrenia.

With this is mind, the first aim of this chapter was to identify the regional white matter abnormalities present in patients with FES, for the purposes of distinguishing between the validities of the aforementioned theories of neural disconnectivity. The second aim of this chapter was to compare the regions affected by white matter atrophy (or hypertrophy) in the FES patients to the regions affected by grey matter atrophy (or hypertrophy) in the

same patients in Chapter 3. By doing this I hoped to gain an insight in to the mechanisms underlying the structural brain abnormalities present in patients with FES.

This chapter has been published *American Journal of Psychiatry*, as an article entitled: "Volumetric white matter abnormalities in first-episode schizophrenia: a longitudinal, tensor-based morphometry study" (see Whitford et al., 2007b).

4.2 ABSTRACT

While schizophrenia has long been considered a disorder of brain connectivity, relatively few studies have investigated for white matter (WM) abnormalities in the disease. Forty-one patients with first-episode schizophrenia (FES) underwent a T1-weighted structural MRI scan within three months of their first presentation to mental health services with psychotic symptoms. Forty-seven age and sex matched healthy controls were scanned for comparison. A first-episode design was employed as it minimized the confounds associated with illness chronicity and long-term exposure to neuroleptic medication. Of the baseline participants, 25 FES patients and 26 controls returned 2-3 years later for a follow-up scan. Voxel-based morphometry in SPM2 was used to identify regional volumetric WM differences between the groups at baseline, while tensor-based morphometry was used to identify the longitudinal changes over the follow-up interval. The FES patients exhibited volumetric deficits in the WM of the frontal and temporal lobes at baseline, as well as volumetric increases in the WM of the fronto-parietal junction bilaterally. Furthermore, the FES patients lost considerably more WM over the

follow-up interval than did the controls in the middle and inferior temporal cortex bilaterally. While there is substantial evidence for abnormal myelination being responsible for the WM irregularities in patients with schizophrenia, I propose that the longitudinal WM reductions observed in this study could have resulted from the death of neurons in the temporal cortex over the follow-up interval, possibly due to a dysfunction in the dramatic period of brain development typically associated with healthy adolescence.

4.3 INTRODUCTION

A dysfunction in neural connectivity has been proposed as being the fundamental abnormality underlying schizophrenia (Andreasen et al., 1999; Friston, 1998; McGuire & Frith, 1996). Given that white matter (WM) tracts constitute the anatomical infrastructure for neural connectivity, it is reasonable to hypothesise the existence of WM abnormalities in patients with schizophrenia. In spite of this, however, relatively few studies have investigated for WM abnormalities in the disease. The aim of this study was to examine for evidence of volumetric WM abnormalities in patients with schizophrenia, both at the time of their first presentation to mental health services with psychotic symptoms, and longitudinally over the first 2-3 years of illness.

Volumetric WM reductions, particularly in the frontal lobe, have been consistently reported in patients with schizophrenia (see Davis et al., 2003 for a review). This volumetric reduction could be indicative of decreased axonal myelination, or axonal

elimination resulting from neuronal death. Furthermore, there is some evidence suggesting that this WM atrophy is progressive over the course of the disease (Velakoulis et al., 2002). A common feature of the majority of previous studies is that they have focused on chronically ill patients – very few studies have investigated WM abnormalities in patients experiencing their first episode of schizophrenia (FES). This is an issue that requires addressing, as investigating the structural underpinnings of schizophrenia early in its course gives significant insight into the nature of the disease, its origins, its clinical course and the optimal path for therapeutic intervention. Furthermore, the first-episode design minimises the confounds associated with long-term exposure to neuroleptic medication, which has been suggested to affect brain structure in and of itself (Lieberman et al., 2005; Madsen et al., 1999). Previous studies that have investigated evidence of WM irregularities in FES patients have produced equivocal results. For example, while several studies have reported WM abnormalities in FES, including impaired myelination of the corpus callosum (Flynn et al., 2003), irregular shape of the corpus callosum (Frumin et al., 2002), reduced fractional anisotropy in fronto-temporal WM (Szeszko et al., 2005) and a decreased magnetization transfer ratio (a putative measure of myelination) in the fasiculus uncinatus (Bagary et al., 2003), others have failed to observe any WM abnormalities (Hirayasu et al., 2001; Salokangas et al., 2002; Cahn et al., 2002a). Inconsistent results have also been reported in the very few studies that have investigated longitudinal WM changes in patients with recent-onset schizophrenia. For example, while Ho et al. (2003) reported progressive atrophy in frontal lobe WM over three years in patients with recent-onset schizophrenia, Rapoport et

al. (1999) did not observe differential rates of WM change over four years between patients with childhood-onset schizophrenia and matched healthy controls.

Aside from the clearly delineated corpus callosum, it is notoriously difficult to manually define WM regions-of-interest (ROIs) consistently between subjects, due to the dearth of referential anatomical landmarks. Hence the majority of previous studies have investigated changes in WM at the level of the whole-brain, or the brain lobe. The lack of sensitivity inherent in such a large-scale analysis may well have contributed to the inconsistency of the previous findings. This is one area in which the automated statistical imaging techniques (e.g. voxel-based morphometry) are particularly advantageous. By investigating for evidence of structural difference at every voxel in the brain, the statistical imaging techniques are able (given appropriate statistical correction for multiple comparisons) to identify small, discrete areas of regional abnormality without requiring the manual tracing of ROIs, which are difficult to define consistently and are necessarily constrained to the regions defined by prior hypotheses.

In this study, voxel-based morphometry was used to investigate for evidence of WM abnormality in patients with first-episode schizophrenia (relative to matched healthy controls) within three months of their first presentation to mental health services with psychotic symptoms (baseline condition). Tensor-based morphometry was used to investigate for evidence of progressive WM atrophy in the FES patients over the first 2-3 years of illness, over and above any corresponding longitudinal changes experienced by the healthy controls (follow-up condition). It was hypothesized that the FES patients

would exhibit frontal, temporal and parietal WM reductions at baseline, relative to the healthy controls, and that these regional abnormalities would degenerate over the follow-up interval, based on the abnormal regional grey matter reductions that I have previously reported in these patients (Chapter 3).

4.4 METHODS

4.4.1 Participants

The participants for both the baseline and follow-up phases of the study were identical to the participants investigated in Chapter 3. A summary of the demographic details of the patient and control samples is provided in Table 3-1. A full description of the recruitment criteria, exclusion criteria, and treatment protocols used for these participants is provided in Section 3.4.1.

4.4.2 MRI Acquisition

The MRI acquisition protocols for the baseline and follow-up phases of the study, for both the FES patients and matched healthy controls, were identical to those employed in Chapter 3. A full description of these acquisition protocols is provided in Sections 3.4.2 and 3.4.3.

4.4.3 Image Pre-Processing

The protocols for image pre-processing were essentially identical to the protocols described in Section 3.4.4 for the baseline study, and Section 3.4.5 for the follow-up study. The only difference between the protocols described in Chapter 3 and the protocols used in this study was that the segmented *white matter* images were pre-processed and used for all analyses in this chapter, while the segmented *grey matter* images were pre-processed and used for all analyses in Chapter 3.

4.4.4 Statistical Analyses

The statistical analyses used for both the baseline and follow-up phases of this study were identical to the analyses described in Sections 3.4.6 and 3.4.7.

4.5 RESULTS

4.5.1 Baseline study

The 41 FES patients showed reduced white matter volume in a number of regions at baseline relative to the healthy controls, after controlling for age, gender, handedness and global WM volume (Figure 4-1 and Table 4-1). These regions of reduction were mainly located in the white matter of the frontal and posterior temporal lobe.

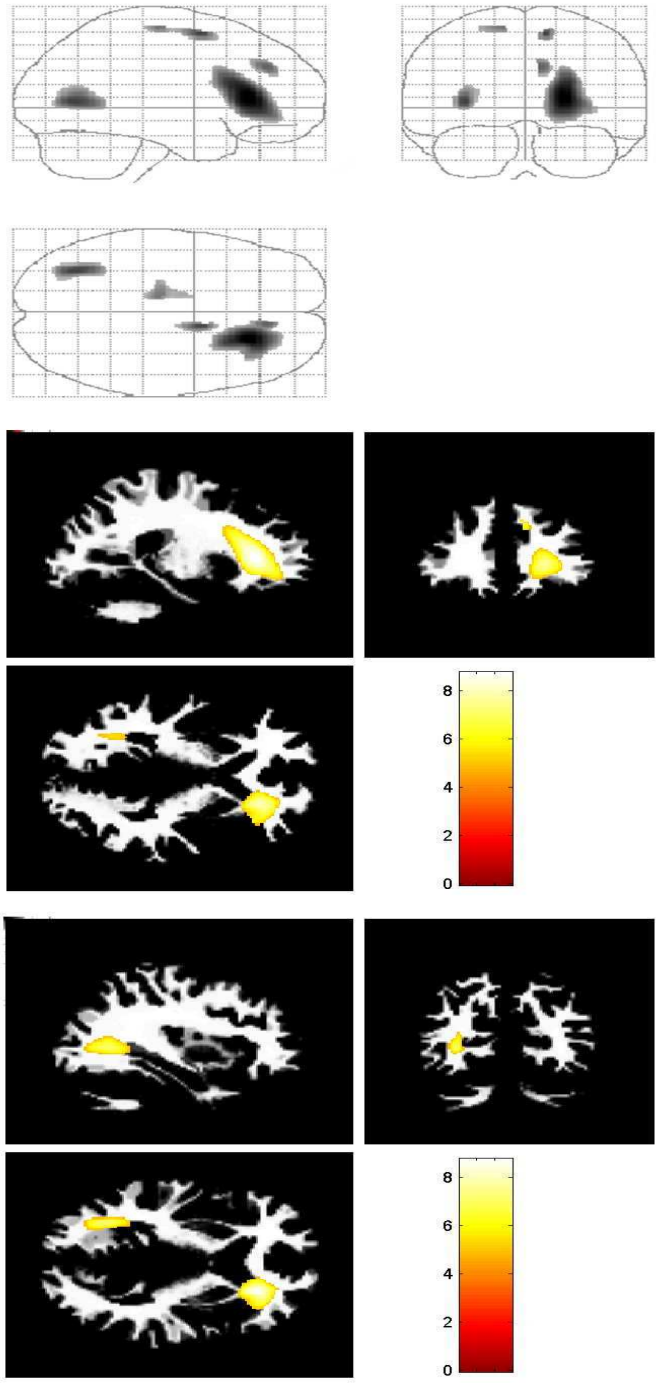

Figure 4-1: Regions of volumetric white matter reduction in 41 patients with first-episode schizophrenia relative to 47 matched healthy controls at the baseline scan. Participants' age, gender, handedness and whole-brain white matter volume were controlled for in the analysis. At the top of the figure, the regions of reduction are displayed as an SPM (P<.05 corrected for multiple comparisons, extent threshold=100 voxels). At the bottom of the figure the regions of reduction are overlaid onto the cleaned, white matter segment of the MNI single-subject T1-weighted MR image.

Anatomical Region	T&T co-ordinate of voxel of maximum significance			T-value	Cluster size
	x	y	z		
Right fronto-occipital fasiculus	21	29	6	8.75	3624
Left inferior longitudinal fasiculus	-33	-64	9	7.24	735
Right cortico-pontine (coticofugal) / thalamocortical (to ventral-anterior nucleus) fibres	11	6	55	7.12	119
Right dorsal medial frontal gyrus	9	39	28	6.52	128
Left pre-central gyrus	-12 -14	-16 -3	61 58	6.1	135

Table 4-1: Baseline Study – regions where the 41 FES patients had less WM at baseline than did the 47 matched healthy controls (statistically controlling for age, gender, handedness and global WM volume)

The FES patients also showed a bilateral region of increased WM volume at baseline relative to the controls at the fronto-parietal junction (Figure 4-2 and Table 4-2).

Anatomical Region	T&T co-ordinates of voxel of maximum significance			T-value	Cluster size
	x	y	z		
Right post-central gyrus	26	-25	34	6.96	1699
	27	-20	26	6.84	
Left dorsal middle frontal gyrus	-32	-13	28	6.43	1046

Table 4-2: Baseline study - regions where the 47 matched healthy controls had less WM at baseline than did the 41 FES patients (statistically controlling for age, gender, handedness and global WM volume)

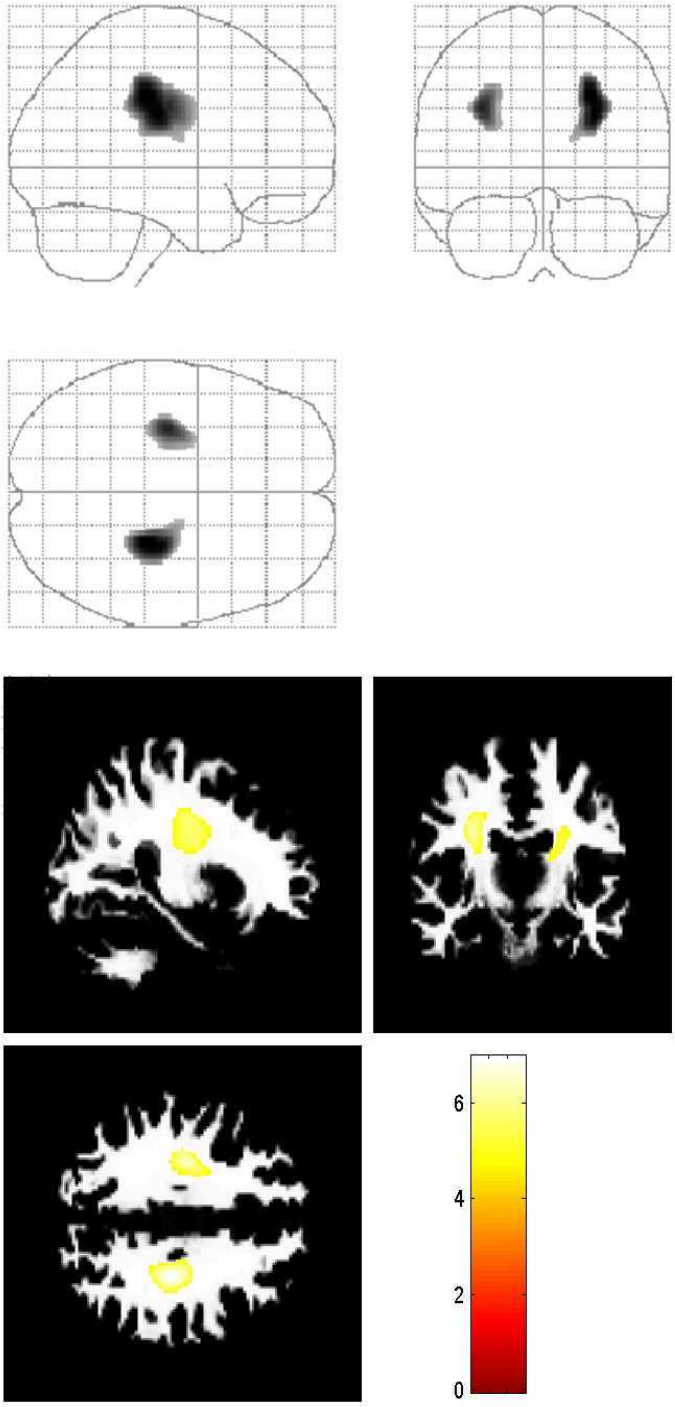

Figure 4-2: Regions of volumetric white matter increase in 41 patients with first-episode schizophrenia relative to 47 matched healthy controls at the baseline scan. Participants' age, gender, handedness and whole-brain white matter volume were controlled for in the analysis. At the top of the figure, the regions of increase are displayed as an SPM (P<.05 corrected for multiple comparisons, extent threshold=100 voxels). At the bottom of the figure the regions of increase are overlaid onto the cleaned, white matter segment of the MNI single-subject T1-weighted MR image.

4.5.2 Longitudinal study

The 25 FES subjects exhibited volumetric WM reductions in the temporal lobe bilaterally over the first 2-3 years of their illness, over and above any longitudinal change experienced by the matched healthy controls (Figure 4-3 and Table 4-3). These regions of longitudinal WM loss were confined to the middle and inferior temporal cortices. There were no regions in which the controls were observed to lose more WM over the follow-up interval than the FES patients.

Anatomical Region	**T&T co-ordinates of voxel of maximum significance**			**T-value**	**Cluster size**
	x	y	z		
Left inferior temporal gyrus	-53	-26	-17	8.02	1190
Left middle temporal gyrus	-53	-36	-13	7.54	
Left posterior middle temporal gyrus	-53	-41	-8	6.92	
Right fusiform gyrus	48	-24	-19	5.39	546
Right anterior middle temporal gyrus	51	-14	-18	5.29	
Right middle temporal gyrus	57	-28	-12	5.27	

Table 4-3: Longitudinal study – regions where the 25 FES patients lost more WM over the follow-up interval than did the 26 matched healthy controls (statistically controlling for age, gender, handedness and FU interval)

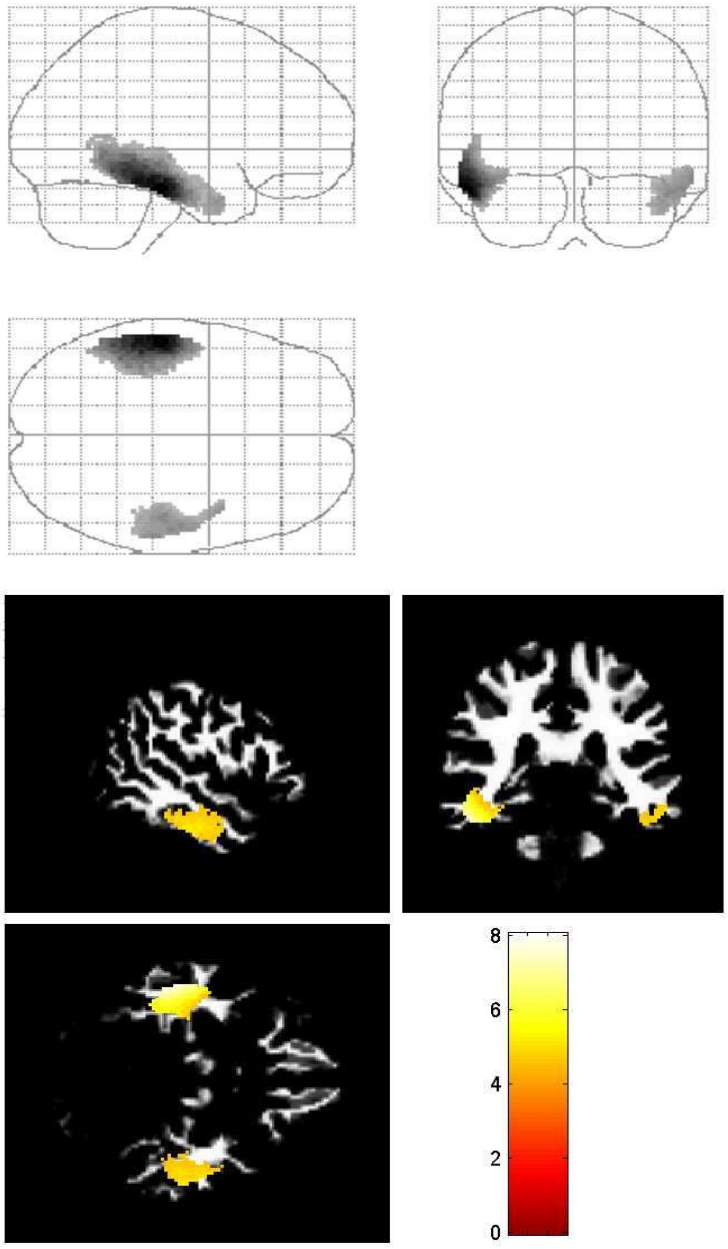

Figure 4-3: Regions where the 25 patients with first-episode schizophrenia who underwent a follow-up scan lost a greater volume of white matter over the follow-up interval compared to the 26 matched healthy controls who underwent a follow-up scan. Participants' age, gender, handedness and follow-up interval were controlled for in the analysis. At the top of the figure, the regions of reduction are displayed as an SPM (P<.05 corrected for multiple comparisons, extent threshold=100 voxels). At the bottom of the figure the regions of reduction are overlaid onto the cleaned, white matter segment of the MNI single-subject T1-weighted MR image.

4.6 DISCUSSION

Schizophrenia has long been thought of as a disorder of brain connectivity (Friston, 1998). In spite of this, however, relatively few studies have investigated for white matter abnormalities in patients with schizophrenia, and the majority of those that have focused on patients with chronic schizophrenia, and hence have suffered from the confounds associated with illness chronicity and long-term exposure to neuroleptic medication. In this study, voxel-based morphometry (VBM) was used to identify widespread reductions in WM volume in patients with first-episode schizophrenia at the time of their first presentation to mental health services with psychotic symptoms, relative to matched healthy controls (Figure 4-1 and Table 4-1). The fact that these reductions were most prominent in the frontal and temporal lobes is consistent with theories which argue that a disintegration of fronto-temporal connectivity underlies a number of the 'positive' symptoms of schizophrenia, with disorganized thinking being the most salient example (Hoffman & McGlashan, 2001), and auditory hallucinations (Frith, 1992) and certain delusions (e.g. delusions of alien control) (Frith & Done, 1989) also being implicated. A failure to recognise one's self-generated thoughts and actions as being self-generated has been proposed as the general mechanism underlying these symptoms of reality distortion. Frith et al. (2000) considered the example of moving one's hand. Signals arising in the supplementary motor area (SMA; where actions are initiated) project to both a) the parietal lobe – which is involved in generating a conscious awareness of the intention to move the hand, and b) the primary motor area (PMA) – which projects down the spinal column in order to actually move the hand. Frith et al. (2000) suggested that the common

schizophrenic delusion of alien control (the belief that one's body is being controlled by an external agent) could arise because of dysfunctional connectivity between the SMA and the parietal lobe, such that the patients' conscious awareness of their intention to move arose after their movement had already occurred. I propose that such a dysfunction could potentially arise from either 1) an abnormally slow connection between the SMA and the parietal lobe – which could correspond to decreased regional WM volume, particularly if it is due to decreased myelination as described below, or 2) an abnormally fast connection between the SMA and the PMA – which could correspond to increased regional WM volume, such as was observed in the frontal lobe WM in the FES patients at baseline (Figure 4-2 and Table 4-2). Obviously this model is largely speculative and would require a great deal more empirical investigation before it would be possible to be confident of its validity.

Several lines of evidence have indicated that abnormalities in axonal myelination underlie the WM abnormalities observed in schizophrenia (see Davis et al., 2003 for a review). Patients with schizophrenia have been observed to show WM hyperintensities on T2-weighted MR images (Sachdev & Brodaty, 1999), which are associated with demyelination (Drayer, 1988). They also show reductions in the magnetization transfer ratio which is a putative measure of myelination (Foong et al., 2000; Bagary et al., 2003), and reductions in fractional anisotropy using diffusion tensor imaging (Kubicki et al., 2005; Szeszko et al., 2005), which has been associated with decreased axonal integrity, and has been found to be reduced in patients with the demyelinating disease multiple sclerosis (Filippi, Cercignani, Inglese, Horsfield, & Comi, 2001). Furthermore, an

immunohistochemical study reported abnormally reduced numbers of oligodendrocytes (glial cells involved in the production and maintenance of myelin in the CNS) in the WM of the superior frontal gyrus in patients with schizophrenia *post mortem* (Hof et al., 2003). Finally, a genetic study using DNA microarray analysis reported five genes implicated in the formation and maintenance of myelin sheaths to be down-regulated in patients with schizophrenia relative to healthy controls (Hakak et al., 2001). Thus there is substantial evidence indicating that abnormal myelination underlies the WM abnormalities reported in schizophrenia. However a second, not mutually exclusive, possibility is that the WM loss reflects the elimination of axons resulting from neuronal death in patients with schizophrenia. I suggest that neuronal death, rather than demyelination *per se*, may underlie the longitudinal WM reductions observed in the FES patients in this study (Figure 4-3 and Table 4-3). In Chapter 3, when investigating the same subjects, I reported longitudinal reductions in temporal lobe grey matter in the FES patients over and above the reductions experienced by the healthy controls over the follow-up interval. As can be seen in Figure 4-4, these longitudinal GM reductions were immediately lateral to the longitudinal WM reductions observed in this study, particularly in the right hemisphere.

Given that the cortex is largely constituted of pyramidal neurons which project into the adjacent WM (Niedermeyer et al., 1999), it is tempting to speculate that the longitudinal WM reductions reported in this study were caused by the death of the pyramidal neurons in the temporal cortex. Contrary to this speculation, however, the majority of previous studies have not found evidence of a large-scale elimination of cortical neurons in

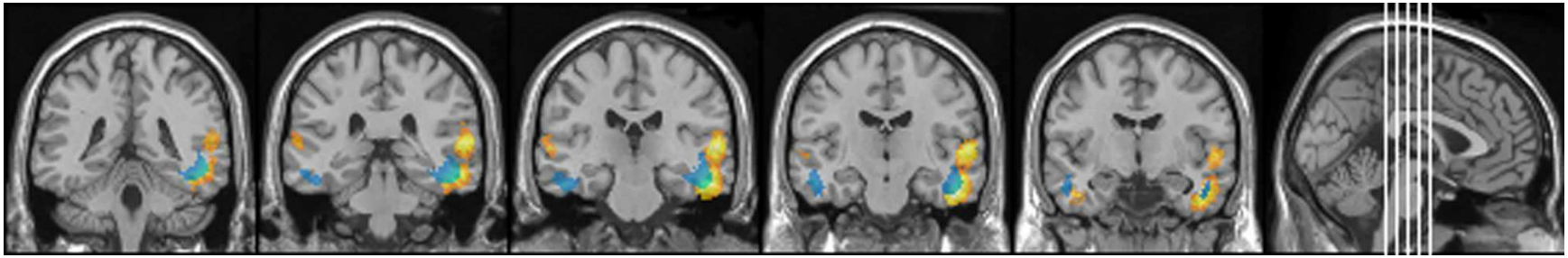

Figure 4-4: Regions where the 25 patients with first-episode schizophrenia who underwent a follow-up scan experienced greater volumetric grey matter loss (in yellow) and white matter loss (in blue) over the follow-up interval compared to the 26 matched healthy controls who underwent a follow-up scan

patients with schizophrenia (e.g. see Pakkenberg, 1993). This has led to suggestion that the GM reductions characteristic of schizophrenia are due primarily to the elimination of neuropil (i.e. dendrites and their associated axon terminals), rather than the elimination of neurons *per se* (Selemon et al., 1999). What follows is a brief speculation as to the mechanisms underlying the GM reductions characteristic of schizophrenia, and how they relate to the longitudinal WM reductions observed in this study:

Adolescence and early adulthood is a period of enormous structural change in the healthy human brain (see Chapter 1). Previous studies indicate that this period is associated with a dramatic reduction in the number of cortical synapses and their associated neuropil (Bourgeois & Rakic, 1993; Huttenlocher et al., 1997). The mechanism behind this 'synaptic prune' is not clear, but may involve the targeted elimination of synapses via a similar mechanism to that proposed by Purves (1998) to explain the development of the perinatal nervous system. Purves (1998) has argued that neurons synthesize a limited amount of trophic factors, such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF). In response to stimulation by the pre-synaptic neuron, these trophic factors are expelled into the synapse by the post-synaptic cell and are transported retrogradely to the pre-synaptic cell. Purves (1998) argued that these trophic factors are required for the growth and survival of dendrites and axon-terminals on the pre-synaptic neuron. Synapses that receive a sufficient amount of these trophic factors are maintained, while synapses that receive an insufficient amount are eliminated. Furthermore, Purves (1998) argued that a minimum amount of trophic factor was required to prevent the death

of not only the neuropil on the pre-synaptic neuron, but of the neuron itself, possibly through apoptotic mechanisms (Henderson, 1996). If the 'synaptic prune' in the brains of healthy adolescents is triggered by a sudden reduction in the amount of available trophic factors, possibly triggered by the hormonal changes associated with puberty (e.g. see Forger & Breedlove, 1986; Hendry, 1975), then it is feasible that schizophrenia (with its most common age-of-onset in late adolescence) may be triggered by an abnormally large reduction in trophic factors. Such a reduction in trophic factors could result in an over-vigorous 'synaptic prune' (as has been suggested previously e.g. Feinberg, 1982), and reflected in the abnormally decreased GM volumes typically associated with FES. Evidence supporting this hypothesis comes from recent studies that have observed decreased plasma levels of NGF (Bersani et al., 1999) and elevated plasma levels of autoantibodies to NGF (Klyushnik et al., 1999) in patients with schizophrenia. Regarding the patients in this study, I hypothesize that at some time over the follow-up interval, the amount of trophic factor available to the temporal lobe neurons in the FES patients became inadequate for their survival. This led to the elimination of the relevant neuron bodies and their associated neuropil, which was reflected in the longitudinal grey matter reductions observed in the temporal lobe, as reported in Chapter 3. The death of these neuron bodies meant the elimination of their myelinated axons, which was reflected in the longitudinal WM reductions in the FES patients reported here.

It should be emphasized that while the microscopic mechanisms that have been proposed to underlie the grey and white matter abnormalities observed in the FES patients (i.e. apoptosis, synaptic pruning, demyelination etc), are plausible and were derived from an

analysis of the molecular-level literature (e.g. see Bullmore, Frangou, & Murray, 1997), they are ultimately speculative and untestable with voxel-based morphometry, or MRI more generally for that matter. The grey matter reductions that were, for example, observed in the FES patients could feasibly be caused by a) neuron death (via necrosis or apoptosis), b) the elimination of dendrites and/or dendritic spines, c) the death or mutation of selected glial cells (e.g. astrocytes) or d) one of several other possibilities. Given that the best resolution possible with MRI is approximately $1mm^3$, it is clearly not possible to distinguish between these possibilities on the basis of the MR data *per se*.

Chapter 2 reported evidence of substantial, abnormal GM atrophy in the FES patients, over the 2-3 year follow-up interval. This raises the question of whether the WM abnormalities reported in the present study are secondary to (i.e. resulted from) these GM changes. However this explanation seems unlikely because of the fact that relative to the widespread grey matter atrophy exhibited by the FES patients over the follow-up interval (which engulfed most of the parietal cortex), there was comparatively little WM atrophy over the same period, and it was confined to the inferior temporal lobe. Given that oligodendrocytes (which constitute the bulk of the WM) depend on their corresponding neuronal axons for survival (Raff et al., 1994; Barres, Jacobson, Schmid, Sendtner, & Raff, 1993), the fact that we observed widespread progressive grey matter loss but relatively circumscribed WM loss suggests that the FES patients did not experience widespread neuron death over the follow-up interval (the aforementioned speculation as to the fate of the inferior temporal lobe neurons notwithstanding). This suggestion is consistent with previous stereological studies which have failed to observe a reduction in

neuron number in the neocortex of patients with schizophrenia (Pakkenberg, 1993). Instead, the results of the present study are consistent with the "reduced neuropil hypothesis" (Selemon et al., 1999), which argues that the characteristic GM loss exhibited by patients with schizophrenia is underpinned by a reduction in dendrites, dendritic spines and glial cells (all of which can occur without a corresponding reduction in WM), rather than the death of neurons *per se*.

In summary, in this study patients with schizophrenia were observed to exhibit widespread WM deficits in the frontal and temporal lobes at the time of their first presentation to mental health services with psychotic symptoms, as well as more localized regions of volumetric increase in the frontal lobes. Evidence of progressive WM reductions in the first 2-3 years of illness was also observed in these patients, in a circumscribed region of the temporal lobe bilaterally. I have suggested that while there is substantial evidence indicating that abnormalities in myelination are responsible for the WM anomalies in patients with schizophrenia, the longitudinal WM reductions reported here could result from the death of neurons in the temporal cortex, possibly due to a dysfunction in the dramatic period of brain development typically associated with healthy adolescence.

CHAPTER 5

GREY MATTER DEFICITS AND SYMPTOM PROFILE IN FIRST-EPISODE SCHIZOPHRENIA

5.1 PREAMBLE

The existence of grey matter abnormalities in patients with schizophrenia at the time of their first presentation to mental health services (i.e. at baseline) was reported in Chapter 3. The research described in this chapter investigated the nature of the relationship between patients' grey matter atrophy at baseline and their associated symptom profile. To this end, the brain regions where 31 patients with FES exhibited grey matter atrophy at baseline, relative to 30 matched healthy controls, were identified and the volumes of these regions calculated. The volumes of these regions-of-reduction were correlated with patients' scores on three symptom dimensions generated from the scales of the PANSS, namely Psychomotor Poverty, Disorganization and Reality Distortion. Although there have been a number of studies in the literature that have examined the relationship between brain structure and symptom profile in patients with chronic schizophrenia, this study had the advantage of testing the subjects before they had experienced significant exposure to neuroleptic medication, which can obviously influence patients' symptomatology but which has also been suggested to influence their brain structure.

The aim of this study was to identify the structural brain abnormalities associated with differing clinical profiles in patients with FES, with the underlying aim being to elucidate the neurological origins of the various symptoms of schizophrenia.

This chapter has been published in the journal *Psychiatry Research: Neuroimaging* as an article entitled: “Grey matter deficits and symptom profile in first episode schizophrenia” (see Whitford et al. (2005) and Appendix 4).

5.2 ABSTRACT

Several studies have investigated for evidence of grey matter reductions in first episode schizophrenia (FES), but few have examined the relationship between grey matter reduction and clinical profile. A group of 31 patients with strictly defined FES and 30 healthy controls underwent a T1-weighted magnetic resonance imaging (MRI) scan. Voxel-based morphometry in SPM99 was used to identify four distinct regions of grey matter reduction in the FES subjects. These regions-of-interest (ROIs) were in the left ventral prefrontal cortex (ROI 1), left parietal and temporal cortices (ROI 2), right cerebellum (ROI 3), and right frontal and parietal cortices (ROI 4). These ROIs were transformed into binary masks, which were convolved with patients’ pre-processed grey matter images. Patients’ grey matter volumes in these regions were correlated with their composite scores on the following three symptom dimensions: Psychomotor Poverty, Disorganization and Reality Distortion. The volumes of ROIs 1, 2 and 4 were found to be significantly correlated with patients’ Reality Distortion syndrome score. These findings indicate that distinct, widespread grey matter reductions are present very early in the course of schizophrenia. The results also suggest a possible structural underpinning for the abnormal brain activity typically associated with symptoms of Reality Distortion.

5.3 INTRODUCTION

A considerable proportion of the structural magnetic resonance imaging (MRI) research in schizophrenia has focused on identifying evidence of grey matter loss in patients with chronic schizophrenia, and investigating the relationship between regional grey matter volumes and symptom severity in these patients. Reductions in the prefrontal cortex, superior temporal gyrus and medial temporal lobe have been widely reported (Kasai et al., 2002; McCarley et al., 1999; Pearlson et al., 1999). In addition, several studies have identified associations between the volume of the superior temporal cortex and the severity of symptoms of reality distortion (Shapleske et al., 2002; Wright et al., 1995) and between prefrontal cortex volume and symptoms of psychomotor poverty (Chua et al., 1997). Some studies, however, have failed to reproduce these relationships (Paillere-Martinot et al., 2001; Wible et al., 1995).

In comparison to research with chronic patients, substantially less has been written about grey matter loss and structure/symptom relationships in patients with first episode schizophrenia (FES). Yet the investigation of FES is important to elucidating the core pathophysiology of this illness, given that it overcomes the confounds associated with illness chronicity, such as possible progressive grey matter atrophy and prolonged exposure to neuroleptic medication (Madsen et al., 1999). The available FES studies have reported similar patterns of grey matter reduction at the time of patients' first psychotic episode to those observed in chronic patients, including reductions in the prefrontal cortex (Hirayasu et al., 2001), temporal cortex (Hirayasu et al., 1998), medial temporal

lobe (Velakoulis et al., 1999), parietal cortex (Kubicki et al., 2002), and cerebellum (Ichimiya et al., 2001), although some studies have failed to identify these reductions (Molina, Sanz, Sarramea, Benito, & Palomo, 2004). At least two previous studies have investigated the relationship between regional grey matter volumes and symptom severity in first episode patients. In 25 drug-naïve FES patients, Kim et al. (2003) observed a negative association between the volume of the left anterior portion of the superior temporal gyrus and the Reality Distortion syndrome score; assessed by the sum of hallucinations and delusions ratings on the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984b). Conversely, the right posterior portion of the superior temporal gyrus was found to be positively associated with the Negative syndrome score, which was the sum of alogia, affective flattening, avolition-apathy and anhedonia-asociality ratings on the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1984a). In contrast, Fannon et al. (2000) failed to find any relationship between any symptom rating on the Positive and Negative Syndrome Scale and whole brain, global cortex, or temporal lobe grey matter volume in 37 patients with first episode psychosis.

The aim of the present study was to identify regions of grey matter reduction in a stringently defined FES sample, and to investigate whether the severity of this regional grey matter reduction corresponded to the severity of patients' clinical symptoms. Voxel-based morphometry was used to identify the brain regions in which FES patients exhibited reduced grey matter volume relative to matched healthy controls. The volumes of these regions were correlated with patients' scores on three pre-defined syndromes of

Psychomotor Poverty, Disorganization and Reality Distortion, based on Liddle's (1987b) tripartite model of schizophrenic symptomatology. Given the associations observed by Liddle et al. (1992) between these syndromes and patterns of regional cerebral blood flow, it was predicted that Reality Distortion would be associated with related alterations in temporal regions, while Disorganization and Psychomotor Poverty would be associated with reductions in the prefrontal cortex.

5.4 METHODS

5.4.1 Participants

Thirty-one FES patients (20 males, 11 females) were recruited as part of the Western Sydney First Episode Psychosis project. These patients were a subset of the patients described in the 'baseline' studies of Chapters 3 and 4. A stringent criterion for first episode status was employed whereby all patients were recruited within 3 months of their first presentation to mental health services with psychotic symptoms, although some patients had previously presented with symptoms of depression and anxiety that were not judged to be psychotic at the time. Patients' average age at the time of scanning was 19.3 years (SD=3.5). Patients' average estimated duration of untreated psychosis (based on patients' self-report and corroborative familial reports) was 6.4 months (± 8.1). At the time of scanning, 27 patients were being treated with atypical antipsychotic medication while four were neuroleptic-naive. Patients' average chlorpromazine equivalent

medication dosage was 247 mg/day (± 212). At the time of scanning, no patient had been treated for more than 3 months with antipsychotic medication.

Thirty healthy control subjects (20 males, 10 females), matched to patients in age (19.3 years ± 3), were recruited from parallel geographical regions in collaboration with the Brain Resource International Database [http://www.brainresource.com (Gordon, 2003)]. Control subjects were screened for the presence of an Axis-I disorder, using the SPHERE (Hickie et al., 1998) and subjects were also excluded if they reported a first-degree family member with an Axis I diagnosis. It was also determined that the controls were within the normal range on depression, anxiety and stress, assessed using an abbreviated version of the Depression Anxiety Stress Scale (DASS) (Lovibond et al., 1995).

Exclusion criteria for both groups were a past history of substance dependence, exposure to electroconvulsive therapy, mental retardation, neurological disorder including epilepsy, and a history of head injury causing loss of consciousness for at least 1 hour. Pre-morbid IQ estimates were based on the Wide Range Achievement Task Revision 3 (WRAT-3) (Wilkinson, 1993) and the Spot-the- Real-Word Test (Baddeley et al., 1993) and were comparable between the patient (99 ± 10) and control (104 ± 6) groups. After a detailed description of the study, each subject gave written informed consent to participate, in accordance with Australian National Health and Medical Research Council guidelines. All subjects were right-handed.

5.4.2 Clinical assessment

Diagnosis of schizophrenia was made using DSM-IV criteria (American Psychiatric Association, 1994), by a consensus conference of three senior psychiatrists, at least two of whom were independent of the study. The Positive and Negative Syndrome Scale (PANSS) was used to assess patients' symptoms (Kay et al., 1989). Inter-rater reliability on the PANSS between the psychiatrists was above 0.8. As has been described elsewhere (Harris, Williams, Gordon, Bahramali, & Slewa-Younan, 1999), a principal components analysis of the items on the PANSS Positive subscale and the PANSS Negative subscale produced three factors that corresponded to the syndromes of Liddle (1987b), namely Reality Distortion, Psychomotor Poverty and Disorganization. Following the procedure of Liddle (1987a), the factor scores were calculated by summing the appropriate PANSS item scores. The following items were combined for each syndrome: Reality Distortion (sum of delusions, hallucinatory behaviour, suspiciousness and hostility symptom item ratings, group mean=11.5 ± .7), Psychomotor Poverty (sum of blunted affect, emotional withdrawal, social withdrawal, poor rapport and lack of spontaneity item ratings, group mean=14.3 ± .7), and Disorganization (sum of conceptual disorganization, grandiosity, excitement, difficulty in abstract thinking and stereotyped thinking item ratings, with group mean=11.6 ± .8).

5.4.3 MRI acquisition and pre-processing

All subjects underwent a single T1 weighted volumetric MPRAGE structural MRI scan on a Siemens 1.5-Tesla Vision Plus system at Westmead Hospital, Sydney. Images were obtained coronally with the following scan parameters: TR=9.7 ms, TE=4 ms, TI=200 ms, flip angle=12º, voxel size=1x1x1 mm, acquisition time=8 min, 26 s.

Images were pre-processed using voxel-based morphometry (VBM) (Ashburner et al., 2000) in SPM99 (Wellcome Department of Cognitive Neurology, London, UK). The voxel-based technique performs a statistical analysis at each voxel in an MR image, and thus does not require the manual tracing of regions of interest, which is time consuming and prone to inconsistencies. Each subject's image was spatially normalised to the Montreal Neurological Institute (MNI) T1-image template. The first step in spatial normalization involved estimating the optimal 12-parameter affine transformation (3 translations, 3 rotations, 3 zooms and 3 shears) for matching the subject's image to the template. The second step accounted for global non-linear shape differences, which were modelled by a linear combination (7x8x7) of smooth spatial basis functions (Ashburner et al., 2000). The normalized images were re-sliced with 1.5-mm^3 voxels before being segmented into grey matter, white matter and cerebrospinal fluid (CSF) probability maps. Segmentation was based on each voxel's signal intensity and an a priori expectation of the anatomical location of the different tissue types. After segmentation, the images were stripped of extra-cerebral voxels using the 'Xtract brain' render function in SPM99. To adjust for the growth and shrinkage of voxels that can occur during spatial normalisation,

voxel values in the cleaned, segmented images were modulated with the Jacobian determinants derived from the spatial normalization (Good et al., 2001). Thus if a grey matter voxel doubled in size as a result of normalization, SPM halved its grey matter probability value for the purposes of calculating its grey matter volume (Ashburner et al., 2001). The grey matter images (on which all analyses were performed) were smoothed with a Gaussian kernel of 12-mm full-width at half-maximum (Job et al., 2002; Kubicki et al., 2002).

5.4.4 Statistical analysis

5.4.4.1 Regional grey matter volume reductions in FES patients relative to controls:

Statistical analyses (which can be regarded as analyses of covariance; ANCOVAs) (Friston et al., 1995) were undertaken in SPM99 to identify the brain regions where FES patients exhibited grey matter volume reductions relative to the healthy controls. Global grey matter volume (calculated by summing the voxel values in each subject's pre-processed grey matter image) was included as a nuisance covariate in the analysis. Output was in the form of Statistical Parametric Maps (SPMs), based on a voxel-level height threshold of $P < 0.05$ (corrected for multiple comparisons using Gaussian random field theory; Worsley et al., 1996) and a cluster-level extent threshold of 400 contiguous voxels that corresponded to approximately 1.35 cubic centimetres of tissue. Coordinates for foci of maximal grey matter change within each supra-threshold cluster were produced as MNI coordinates. To facilitate interpretation of results relative to previous studies, these MNI coordinates were transformed into Talairach (Talairach et al., 1988)

coordinates using the 'mni2tal.m' Matlab script written by Matthew Brett (http://www.mrccbu.cam.ac.uk/Imaging).

5.4.4.2. Relationship between regional grey matter volume and syndrome scores in the FES patients: SPM99 was used to extract the grey matter volumes for the above regions of reduction (given the extent threshold of 400 contiguous voxels). These regions were extracted as grey-scale images in MNI space using the 'Write Filtered' option on the SPM99 graphic user interface. The grey-scale images were transformed into binary images using the image calculator in SPM99. These binary images were convolved with all subjects' pre-processed grey matter images, and the volumes of these regions of interest (ROIs) were calculated by summing the constituent modulated voxel values. Exploratory partial correlation analyses in SPSS v.10.0 were used to investigate the relationship between patients' syndrome scores (Psychomotor Poverty, Disorganization and Reality Distortion) and their ROI grey matter volumes. Patients' gender, estimated duration of untreated psychosis and chlorpromazine equivalent medication dosages were statistically controlled for in the analyses.

5.5 RESULTS

5.5.1 Regional grey matter volume reductions in FES patients relative to controls

There were four regions in which the FES subjects exhibited reduced regional grey matter volumes relative to healthy controls (Table 5-1; Figure 5-1). These ROIs were defined as follows: ROI 1 was located in the left prefrontal cortex, with cluster centres in the superior frontal gyrus and medial frontal gyrus. ROI 2 was located in the left temporal and parietal cortex, and extended from cluster centres in the superior temporal gyrus and postcentral gyrus, to the supramarginal gyrus (Talairach: -62 -45 32) and inferior parietal lobule (Talairach: -47 –46 49). ROI 3 was confined to the right cerebellum while ROI 4 was located in the right frontal and parietal cortex, and extended from cluster centres in the pre- and post-central gyri to the middle frontal gyrus (Talairach: 59 29 23) and inferior frontal gyrus (Talairach: 50 27 -11). In addition to these regional reductions, the FES patients were observed to have a significantly reduced whole-brain grey matter volume compared with the control subjects [t(59)=2.2922, P=0.005].

ROI	Anatomical Label	Cluster centres (local maxima > 8MM apart per cluster)	Cluster centres: Talairach coordinate of voxel of maximum significance			Cluster size
			x	*y*	*z*	
ROI 1	Left ventral prefrontal cortex	Superior frontal gyrus	-29	52	-3	2038
		Medial frontal gyrus	-9	46	6	
		Medial frontal gyrus	-9	41	14	
ROI 2	Left parietal and temporal cortex	Superior temporal gyrus	-65	-27	14	3518
		Postcentral gyrus	-55	-18	45	
		Postcentral gyrus	-59	-24	40	
ROI 3	Right cerebellum	Cerebellum	29	-50	-32	492
		Cerebellum	33	-59	-32	
		Cerebellum	44	-45	-28	
ROI 4	Right frontal and parietal cortex	Postcentral gyrus	56	-12	45	4101
		Postcentral gyrus	64	-10	19	
		Precentral gyrus	61	3	16	

Table 5-1: Descriptive details of the four regions of grey matter volume reduction in 31 patients with first episode schizophrenia relative to 30 matched healthy controls ($P < 0.05$ corrected for multiple comparisons, extent threshold=400 voxels)

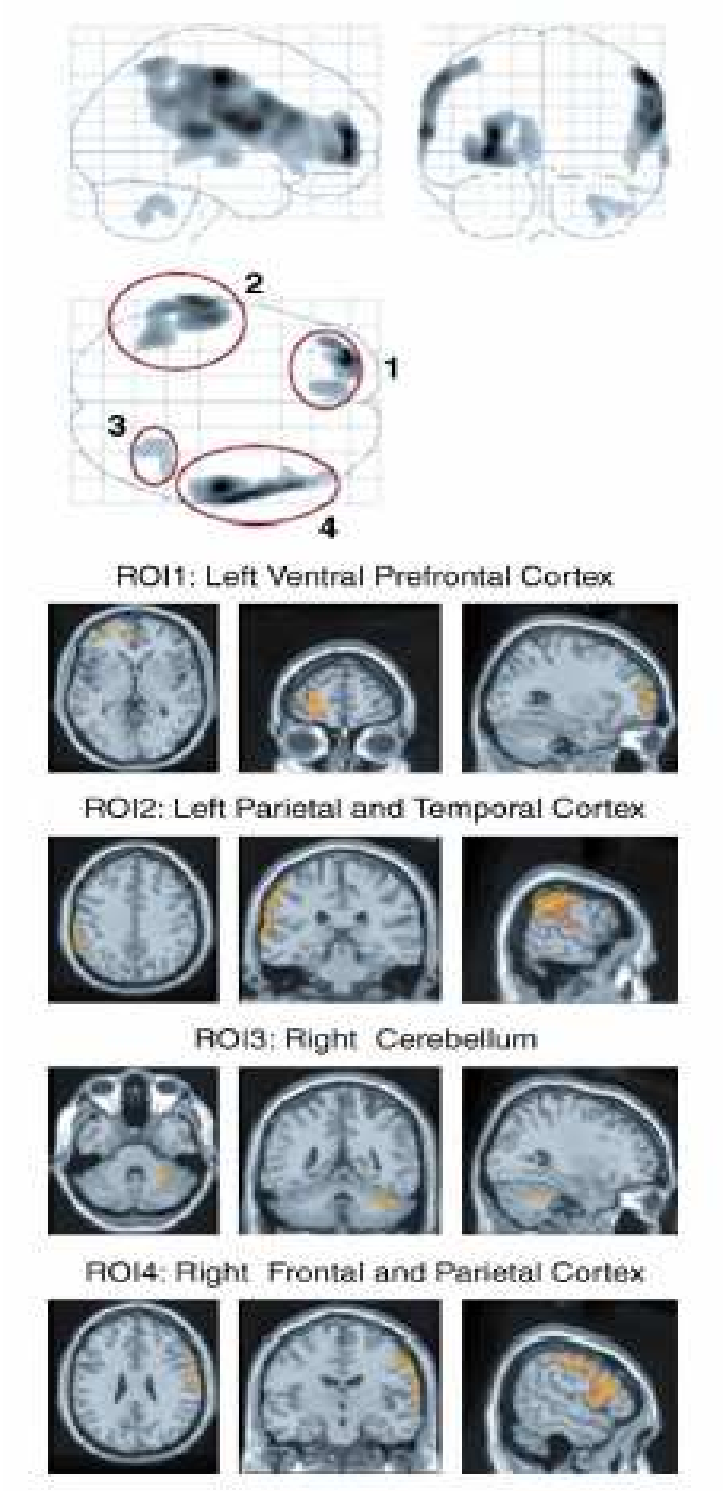

Figure 5-1: Four regions of grey matter volume reduction in 31 patients with first episode schizophrenia relative to 30 matched healthy controls. The regions of reduction are displayed as an SPM ($P < 0.05$ corrected for multiple comparisons, extent threshold=400 voxels). Slices of the masks created from these regions are overlaid onto the MNI single-subject T1-weighted MR image.

To validate the VBM results, the left superior temporal gyrus was manually traced on all subjects in 'MRICRO' (Chris Rorden, http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html) (Figure 5-2), and its volume compared between the FES and control groups. The left STG was chosen due to its being a distinct and clearly delineated structure that was found to be substantially reduced in the FES patients in the VBM analyses (Table 5-1). An independent samples *t*-test revealed the FES group to have a reduced left STG volume relative to the matched control group [t(59)=2.161, P=0.035], a result that confirmed the VBM findings.

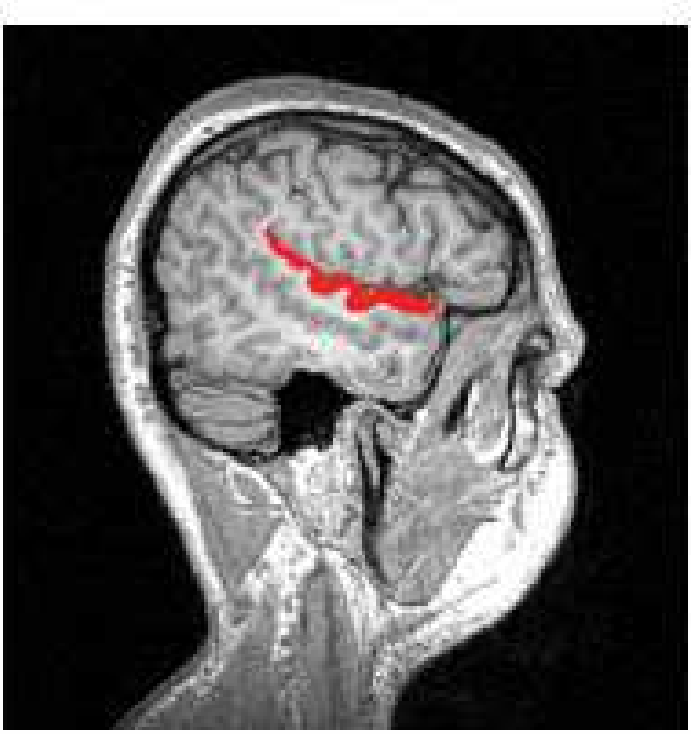

Figure 5-2: To validate the VBM results, the left superior temporal gyrus was manually traced (as illustrated) and its volume calculated in MRICRO for all 31 FES and 30 matched control subjects. The left superior temporal gyrus was chosen due to its being a distinct and clearly delineated structure that was found to be substantially reduced in the FES patients in the VBM analysis.

5.5.2 Relationship between regional grey matter volume and syndrome scores in the FES patients

After controlling for gender, estimated duration of untreated psychosis and chlorpromazine-equivalent medication dosage, the Reality Distortion (RD) syndrome score was found to be positively correlated with the grey matter volume of ROI 1 (partial r =0.4386, p=0.028), ROI 2 (partial r =0.5346, p=0.006) and ROI 4 (partial r =0.5263, p=0.007). As patients with severe RD symptoms could feasibly have been treated earlier and with higher medication dosages than patients with milder RD, an outcome that could confound the analyses, patients' estimated duration of untreated psychosis and chlorpromazine medication dosage were removed from a subsequent set of analyses. The correlations remained statistically significant after removing these two covariates (ROI 1: partial r = 0.3691, p=0.045; ROI 2 partial r =0.3638, p=0.048; ROI 4: partial r=0.3711, p=0.044).

The positive correlation between RD syndrome score and the volumes of ROI 1, ROI 2 and ROI 4 suggested that patients with high RD syndrome scores had larger ROI volumes than did patients with low RD syndrome scores. This raised the question of whether patients with high RD syndrome scores had *abnormally* high ROI 1, 2 and 4 volumes (i.e. relative to healthy controls). To address this possibility, independent samples t tests were used to compare the volumes of these three regions in the 30 control subjects with those in the 19 FES patients with the highest RD syndrome scores (RD syndrome score >12). The results showed that the FES patients with high RD syndrome scores still had

significantly reduced ROI 1 [$t(47)=5.755$, $p<0.001$], ROI 2 [$t(47)=5.582$, $p<0.001$] and ROI 4 [$t(47)=5.164$, $p<0.001$] volumes compared with the control subjects. Thus, while patients with high RD scores had larger ROI 1, 2 and 4 volumes than did patients with low RD scores, these regions were still significantly reduced relative to the matched healthy controls.

5.6 DISCUSSION

In this study, voxel-based morphometry was used to identify the regions of reduced grey matter volume in a stringently defined FES group relative to matched healthy controls. The relationship between these regional reductions and patients' clinical symptoms (in terms of the three syndromes of Reality Distortion, Psychomotor Poverty and Disorganization) was also investigated. FES patients exhibited grey matter reductions in the following four distinct regions: ROI 1 was located in the left ventral prefrontal cortex, ROI 2 was located in the left temporal and parietal cortices, ROI 3 was located entirely in the right cerebellum, and ROI 4 was located in the right frontal and parietal cortices (see Figure 5-1 and Table 5-1 for details). The volumes of ROI 1, ROI 2 and ROI 4 were also found to be positively correlated with Reality Distortion syndrome score in the FES patients.

The regional grey matter reductions observed in this study are broadly consistent with those reported in previous structural MRI studies of FES, which have used various analytic techniques. Grey matter reductions in the temporal cortex (in particular, the

superior temporal gyrus) have consistently been reported in FES (Hirayasu et al., 1998; Keshavan et al., 1998; Kim et al., 2003). Frontal volume reductions have also been commonly reported, including in the anterior cingulate gyrus (Kubicki et al., 2002), superior frontal gyrus (Job et al., 2002) and inferior frontal gyrus (Pantelis et al., 2003). The consistency of these findings has led some researchers to argue that structural abnormalities in the frontal and temporal cortices are responsible for the neurochemical abnormalities that underlie the symptoms of psychosis in patients with schizophrenia (Gray, 1998).

The observation of grey matter reductions in the cerebellum (ROI 3) has previously been reported in FES (Ichimiya et al., 2001). Whilst the cerebellum has traditionally been thought to be exclusively responsible for motor coordination, a number of studies have implicated it in the higher cognitive processes typically dysfunctional in schizophrenia (Kim, Ugurbil, & Strick, 1994; Middleton & Strick, 1994). Indeed, Andreasen et al. (1999) have argued that the fundamental cognitive deficit in schizophrenia is a disruption in the coordination of mental activity that is due to a breakdown in a feedback loop passing through the cerebellum.

In addition to the regional grey matter reductions observed in ROI 1, ROI 2 and ROI 4, the volume of these regions were positively correlated with Reality Distortion syndrome scores in the FES patients. Symptoms of Reality Distortion have most commonly been associated with functional abnormalities in the temporal cortex (Guillem et al., 2003; Kaplan et al., 1993). The superior temporal gyrus has been associated in particular with

auditory hallucinations (McGuire et al., 1996; Shergill et al., 2003). Furthermore, abnormal activations in the inferior parietal cortex have been found to be associated with hallucinations (Shergill et al., 2000) and passivity delusions (Spence et al., 1997), while hypoperfusion in the prefrontal cortex has also been associated with symptoms of Reality Distortion (Sabri et al., 1997). The results of this study suggest that the regional functional abnormalities that have been associated in the literature with Reality Distortion may have structural underpinnings observable with structural MRI.

Three possible accounts were considered as to why the observed correlations between Reality Distortion syndrome score and the grey matter volumes of ROI 1, 2 and 4 were positive in sign. A number of previous studies have reported greater regional grey matter volumes with greater symptom severity in schizophrenia. For example, Chua et al. (1997) reported a positive correlation between medial temporal volume and symptoms of disorganization, while Kim et al. (2003) reported a positive relationship between the volume of the right posterior superior temporal gyrus and negative symptoms. Both these studies suggested that some brain regions might be abnormally enlarged in schizophrenia, possibly due to defective neuronal pruning. Evidence of defective neural pruning in schizophrenia might explain why some studies have found symptoms of Reality Distortion to be associated with functional brain hyperactivity (Spence et al., 1997). However, changes in pruning are unlikely to account for the present findings, given that while patients with high Reality Distortion scores had significantly larger regional volumes than patients with low Reality Distortion scores, they had significantly smaller regional volumes relative to the matched controls. A second possibility is that ‘paranoid’

schizophrenia, defined by symptoms of Reality Distortion, represents an etiologically distinct syndrome of schizophrenia with relatively preserved grey matter compared to patients with other syndromes, if not controls. This proposal accords with evidence for a substantially better prognosis in paranoid schizophrenia than in those classified as suffering from hebrephrenic or undifferentiated schizophrenia (Fenton et al., 1991; Kendler, McGuire, Gruenberg, & Walsh, 1994), and it is more broadly consistent with models positing a heterogeneous course of structural brain atrophy in schizophrenia (DeLisi, Sakuma, Maurizio, Relja, & Hoff, 2004). Thirdly, it is possible that excessive structural atrophy may in fact preclude the formation of hallucinations or highly systematized delusions that would rate highly on the PANSS (Menon et al., 1995), while a degree of grey matter atrophy in select brain systems may be necessary for the formation of Reality Distortion symptoms. This third possibility is discussed in greater detail in Chapter 7.

The use of standardized masks to extract brain regions in order to calculate their grey matter volume was one of the distinguishing features of this study. As the masks used in this study were automatically generated as the regions (in stereotactic space) of grey matter volume reduction in the FES subjects, their use avoided the subjectivity typically associated with manually defined ROIs. However, a difficulty associated with automated masking is that the brain region enclosed by a standardized mask can differ from subject to subject, given normal variations in brain architecture. Although spatial normalization aims to reduce global differences in brain shape, it is imperfect and requires the warping of voxels that can affect the anatomical consistency of the masked region between

subjects. Given that small masks have a higher chance of not enclosing significant proportions of their targeted ROI than large masks, this issue was addressed by setting the cluster-level extent threshold at 400 contiguous significant voxels, corresponding to approximately 1.35 cubic centimetres of tissue, the aim being to create masks of sufficient size to be robust to the aforementioned complications.

In summary, in this study, voxel-based morphometry was used to identify four distinct regions of grey matter reduction in 31 patients with first episode schizophrenia relative to 30 matched healthy controls. The grey matter volumes of three of these regions were found to be positively correlated with patients' Reality Distortion syndrome scores. These results indicate that distinct, widespread grey matter reductions are present very early in the course of schizophrenia, and suggest a possible structural underpinning for the abnormal brain activity typically associated with symptoms of Reality Distortion.

CHAPTER 6

LONGITUDINAL CHANGES IN REGIONAL GREY MATTER VOLUME AND CORRESPONDING EEG POWER IN FIRST-EPISODE SCHIZOPHRENIA

6.1 PREAMBLE

Chapter 2 outlined the relationship between the periadolescent changes in neuroanatomy and the corresponding changes in brain electrophysiology in healthy subjects. It was reported that as the regional grey matter volumes of the four brain lobes decreased curvilinearly between the ages of 10 and 30 years, so did the averaged absolute power of the 'EEG regions' corresponding to these lobes. The study described in this chapter (Chapter 6) used essentially the same methodology to investigate the relationship between neuroanatomy and brain electrophysiology in a subset of 19 patients with first-episode schizophrenia. Unlike in Chapter 2, however, this study employed a longitudinal design in which MRI and EEG data were acquired from the FES subjects at baseline and again 2-3 years later, and the regional changes in GM volume and absolute EEG power calculated for each individual subject.

The rationale for this study was that the observation of an abnormal relationship between the neuroanatomical and electrophysiological changes that occurred over the follow-up interval in the FES patients could provide an insight into the nature of the dysfunctional neural connectivity that has been proposed to be existent in patients with schizophrenia.

This chapter has been published in the journal *NeuroReport* as an article entitled: "Longitudinal changes in neuroanatomy and neural activity in early schizophrenia" (see Whitford et al., 2007a and Appendix 6).

6.2 ABSTRACT

In Chapter 2 it was reported that a developmentally-related reduction in grey matter (GM) volume was associated with a corresponding decrease in absolute EEG power in healthy adolescents, particularly in the slow-wave frequency band. Furthermore, in Chapter 3 it was reported that a periadolescent sample of patients with first-episode schizophrenia (FES) exhibited abnormally accelerated GM loss over the first 2-3 years of their illness. The aim of this study was to investigate whether this accelerated GM loss resulted in a corresponding reduction in EEG power in patients with FES. Structural MRI and resting EEG recordings were acquired from 19 patients with FES, both at baseline (within three months of their first presentation to mental health services) and follow-up (2-3 years later). Grey matter images were segmented out using voxel-based morphometry, before being parcellated into the frontal, parietal, temporal and occipital lobes. Absolute EEG power was calculated in four 'EEG regions', which corresponded to the four MRI regions, for the slow-wave, alpha and beta frequency bands. While GM volume decreased in all four lobes over the follow-up interval, a corresponding decrease in absolute EEG power was not observed. In fact, an almost-significant *negative* correlation was observed between patients' longitudinal GM change in the frontal and parietal lobes and the longitudinal change in slow-wave EEG power for the corresponding cortical regions. If EEG power is considered to be a function of the number of active synapses and the synchrony of synaptic activity, then these results suggest that FES may be associated with abnormally elevated neural synchrony. This

hypothesis is consistent with previous observations of abnormally elevated gamma phase synchrony in patients with schizophrenia.

6.3 INTRODUCTION

The electroencephalographic (EEG) signal is thought to arise primarily from the post-synaptic potentials of cortical pyramidal neurons (Schaul, 1998). In light of this, it is reasonable to expect that changes in cortical anatomy (due to necrosis, apoptosis, synaptic pruning etc.) would result in changes to the EEG signal. In Chapter 2 it was reported that developmentally-related reductions in cortical grey matter (GM) were associated with corresponding reductions in absolute EEG power (particularly slow-wave <7.5Hz) in healthy adolescents. The aim of the study described in this chapter was to investigate whether the accelerated GM loss experienced by patients with first-episode schizophrenia (FES) was associated with a correspondingly accelerated reduction in EEG power. Investigating the relationship between cortical neuroanatomy and EEG signal in patients with schizophrenia not only provides insight into the correlation between the well-documented structural and electrophysiological abnormalities associated with the disease, but also provides insight into the generalisability of models of brain electrophysiology to pathological subjects (e.g. Robinson et al., 2001). The benefits of investigating first-episode patients in particular is that 1) they are known to lose substantial cortical GM in the first few years of illness, and 2) it minimizes the effect of exposure to neuroleptic medication, which has been shown to influence brain structure and function and in and of itself (Madsen et al., 1999; Lieberman et al., 2005). In contrast

to the documented reduction in cortical GM volume observed in FES patients over the first few years following illness onset (Cahn et al., 2002b; Pantelis et al., 2003), most previous studies have reported *increased* EEG power in FES patients, particularly in the slow-wave (Sponheim, Clementz, Iacono, & Beiser, 2000; Omori et al., 1995) and beta (Harris et al., 1997; Begic, Hotujac, & Jokic-Begic, 2000a) frequency bands, although a *reduction* in alpha power is most commonly reported in the literature (Morihisa, Duffy, & Wyatt, 1983).

This study employed a longitudinal design, in which each subject was scanned at two separate time-points on average 2-3 years apart, in order to investigate the changes in GM volume and EEG power that occurred in 19 patients with FES. A longitudinal design is preferable to a cross-sectional design, in which each subject is scanned only once and age-related changes are inferred with regression, in that it minimises the influence of between-subject nuisance covariates, which makes subsequent statistical analysis more sensitive and robust (Kraemer, Yesavage, Taylor, & Kupfer, 2000). It was hypothesized that patients' GM volume would decrease over the follow-up interval, particularly in the frontal and parietal association cortices, which have been found to be extensively remodelled in adolescence in healthy subjects (Sowell et al., 1999). It was not known, however, whether this reduction in GM would be associated with a corresponding reduction in EEG power, as has been previously observed in healthy controls (see Chapter 2), or whether EEG power would increase over time, as previously reported in the literature.

6.4 METHODS

6.4.1 Participants

Nineteen FES patients were recruited as part of the Western Sydney First Episode Psychosis project, a multimodal project investigating the clinical, neuroanatomical, neuropsychological and psychophysiological profiles of young people in western Sydney experiencing their first-episode of schizophrenia (Harris et al., 2005; Fitzgerald et al., 2004). The demographic data for the FES patients is presented in Table 6-1. A stringent criterion for first episode status was employed whereby all patients were recruited within 3 months of their first presentation to mental health services with psychotic symptoms (defined as hallucinations, delusions, formal thought disorder or prominent negative symptoms that persisted for a minimum of 3 days), although some patients had previously presented with symptoms of anxiety and depression that were not judged to be psychotic at the time. FES patients were interviewed and rated by psychiatrists who had reached an acceptable level of inter-rater reliability ($r > .8$) on the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1989). At follow-up, patients exhibited reduced symptom severity compared to baseline as assessed by the PANSS Positive (t=2.9, p=.009), Negative (t=2.6, p=.017) and General (t=3.9, p=.001) symptom scales. There were no significant differences in patients' chlorpromazine equivalent medication dosage between baseline and follow-up. Exclusion criteria were a past history of substance dependence, exposure to electroconvulsive therapy within the past 6 months, mental retardation (estimated pre-morbid IQ <75, based on Wide Range Achievement Task

Revision 3 (WRAT-3) (Wilkinson, 1993) and the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1997)), neurological disorder including epilepsy, a history of head injury causing loss of consciousness for more than 1 hour, and treatment with lithium. After a detailed description of the study, each subject gave written informed consent to participate, in accordance with Australian National Health and Medical Research Council guidelines. This study was approved by the Western Sydney Area Health Service Human Research Ethics Committee.

	Baseline	**Follow-up**
Age-at-scan (years) mean ± SD [range]	19 ± 3 [13-24]	22 ± 3 [15-27]
Gender	13M, 6F	-
Handedness	17R, 2L	-
DUP (months) median [quartiles]	7 [1-12]	-
Medication dosage (mg/day CPZ eqv.) mean ± SD [range]	283 ± 242 [0-667]	268 ± 309 [0-1000]
PANSS Positive mean ± SD [range]	19 ± 6 [10-29]	15 ± 6 [8-32]
PANSS Negative mean ± SD [range]	20 ± 6 [9-30]	15 ± 6 [8-30]
PANSS General mean ± SD [range]	42 ± 10 [29-58]	31 ± 8 [17-50]
IQ estimate mean ± SD [range]	101 ± 11 [79-124]	102 ± 13 [75-122]
Follow-up interval (months) mean ± SD [range]	32 ± 7 [23-42]	-

Table 6-1: Demographic information at baseline for the 19 patients with first-episode schizophrenia.

6.4.2 MR imaging and parcellation

At baseline and again at follow-up, all FEP subjects underwent a single T1-weighted volumetric MPRAGE structural MRI scan on a Siemens 1.5-Tesla Vision Plus system at Westmead Hospital, Sydney. Images were obtained coronally with the following parameters: TR=9.7 ms, TE=4 ms, TI=200 ms, flip angle=12º, FOV=256mm, voxel size=1mm^3. There were no significant upgrades or modifications of the scanner between

the baseline and follow-up stages of the study. To control for scanner drift, phantom data was collected weekly over the follow-up interval, and the scanner calibrated accordingly.

Images were processed using SPM2 (Wellcome Department of Cognitive Neurology, London, UK), running on Matlab 6.5 (MathWorks, Natick, USA). The full details of the processing protocol used in voxel-based morphometry (VBM) are presented elsewhere (Ashburner et al., 2000; Good et al., 2001). Subjects' brain images were firstly spatially normalized to the ICBM 152 template (Montreal Neurological Institute), which approximates Talairach space. The first step in spatial normalization involved estimating the optimum 12-parameter affine transformation (3 translations, 3 rotations, 3 zooms and 3 shears) for matching the subject's image to the template. The second step accounted for global non-linear shape differences, which were modelled by a linear combination (7x8x7) of smooth spatial basis functions (Ashburner et al., 2000). The normalized images were re-sliced with 1.5 x.1.5 x 1.5 mm voxels, before being segmented into GM, white matter (WM) and cerebrospinal fluid (CSF) probability maps, and stripped of extra-cerebral voxels. Segmentation was based on a cluster analysis method that accounted for each voxel's signal intensity, together with an *a priori* expectation of the anatomical location of the different tissue types. In order to adjust for the growth and shrinkage of voxels that can occur during spatial normalisation, voxel probability values in the cleaned, segmented images were modulated with the Jacobian determinants derived from the spatial normalization (Good et al., 2001). Thus if a brain region doubled in size as a result of normalization, the grey matter probability value for this region was halved for the purposes of calculating its volume (Ashburner et al., 2001). The processed GM

images were smoothed with a Gaussian kernel of 12mm full-width at half-maximum (Job et al., 2002), prior to volume calculation.

The pre-processed GM images were then automatically parcellated into regions-of-interest (ROIs) in MNI space. Both the baseline and follow-up GM images were parcellated with the Automatic Anatomical Labelling masks (AAL) (Tzourio-Mazoyer et al., 2002). Four ROIs, corresponding to the four brain lobes (i.e. frontal, temporal, parietal and occipital) were constructed from the AAL masks, which have been described previously in Table 2-2 and Figure 2-1. The grey-scale ROIs were transformed into binary images using the image calculator in SPM2. The binary images were convolved with patients' pre-processed GM images, and volumes of the four ROIs were calculated for both the baseline and follow-up images by summing the constituent modulated voxel values.

6.4.3 Electrophysiological data acquisition and parcellation

Electroencephalography was acquired using an electrode cap with 19 sites placed according to the international 10-20 system (Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, P3, Pz, P4, T3, T4, T5, T6, O1, O2). Horizontal eye movements were recorded with electrodes placed 1cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3 mm above the middle of the left eyebrow and 1cm below the middle of the left bottom eye-lid. The impedance at each site was below 5 kOhms. All potentials were acquired on a SynAmps (Neuroscan, USA) 32 channel DC

system with a gain of 10^4, a digitalisation rate 250Hz and an upper band pass filter set at 50Hz. Data were corrected for eye movement offline (Gratton et al., 1983), and re-referenced to the average of A1 and A2 (earlobes). The EEG data reported and analysed in the present study were recorded during a two-minute interval in which subjects were asked to rest quietly and limit any eye movement. Subjects were required to refrain from caffeine intake and from smoking for at least two hours prior to the EEG testing.

Spectral power estimation was performed by applying a fast Fourier transformation (FFT) on each artefact-free 2-second epoch. The resultant power spectra were averaged separately for each electrode. Power (in μV^2) was then calculated for three frequency bands - slow-wave (0.5-7.5 Hz), alpha (8-12 Hz) and beta (12.5-34.5 Hz) - and logarithmically transformed in order that the values approximated the normal distribution required by parametric statistical methods.

To investigate the relationship between the change in neuroanatomical grey matter volume and the corresponding change in neural activity, four large-scale neural regions were generated ('EEG regions') that corresponded to the four GM ROIs. Neural activity over the frontal, parietal, temporal and occipital lobes was calculated by averaging the standardized power recorded at each electrode site contributing to each region, for slow-wave, alpha and beta activity. A schematic diagram of the electrodes constituting the four 'EEG regions' can be seen in Figure 6-1. The baseline and follow-up EEG data collections were performed using identical equipment and acquisition protocols.

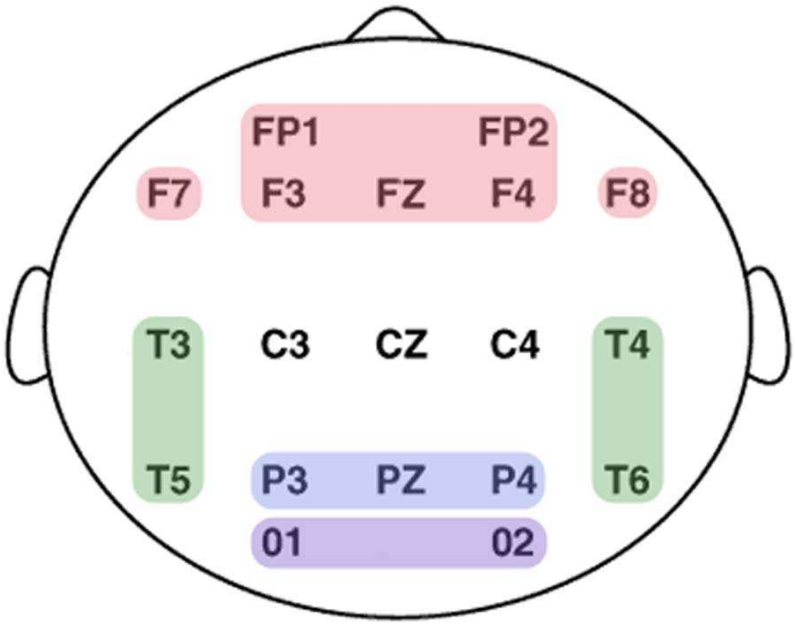

Figure 6-1: Schematic diagram of the constituent electrodes of the four 'EEG regions'. Electrodes making up the frontal lobe 'EEG region' are in red, the temporal lobe 'EEG region' in green, the parietal lobe 'EEG region' in blue and the occipital lobe 'EEG region' in purple. The average power of each 'EEG region' (for each frequency band for each subject) was calculated by averaging the standardized absolute power scores of the constituent electrodes.

6.4.4 Statistical Analysis

Difference scores for the MRI data were calculated by subtracting the GM volume of each subject's masked baseline image from their follow-up image, for the frontal, temporal, parietal and occipital lobes. These GM difference scores were analysed with a paired-samples *t*-test to determine whether the FES subjects experienced a significant change in GM volume over the follow-up interval. Evidence of longitudinal change in the EEG data over the follow-up interval was assessed in the same way. In order to investigate whether longitudinal changes in the GM data could predict longitudinal changes in EEG power, subjects' GM difference scores were correlated with their difference score from the corresponding 'EEG regions'. Age and gender were statistically

controlled for in the correlational analyses, in light of previous research indicating that age and gender affect both GM volume and EEG power (Filipek, Richelme, Kennedy, & Caviness, Jr., 1994; Gasser et al., 1988; Martinovic, Jovanovic, & Ristanovic, 1998; Pfefferbaum et al., 1994).

6.5 RESULTS

The FES patients lost a significant amount of GM volume over the follow-up interval in the frontal lobe ($t(18) = 5.3$, $p<.001$), parietal lobe ($t(18) = 5$, $p<.001$), temporal lobe ($t(18) = 3.5$, $p=.002$) and occipital lobe ($t(18) = 5.1$, $p<.001$) – see Figure 6-2. There was not, however, a corresponding statistically significant decrease in EEG power in any of the four 'EEG regions' in any frequency band. In actual fact, a significant increase in frontal-lobe beta activity ($t(18) = 2.8$, $p=.013$), and in parietal-lobe beta activity ($t(18) = 2.3$, $p=.031$) was observed over the follow-up interval – see Figure 6-3. This finding was consistent with the nearly significant partial correlations that were observed between the frontal GM difference score and the corresponding EEG difference score for the slow-wave band (partial $r = -.48$, $p = .051$), and the parietal GM difference score and the corresponding slow-wave difference score (partial $r = -.47$, $p=.058$).

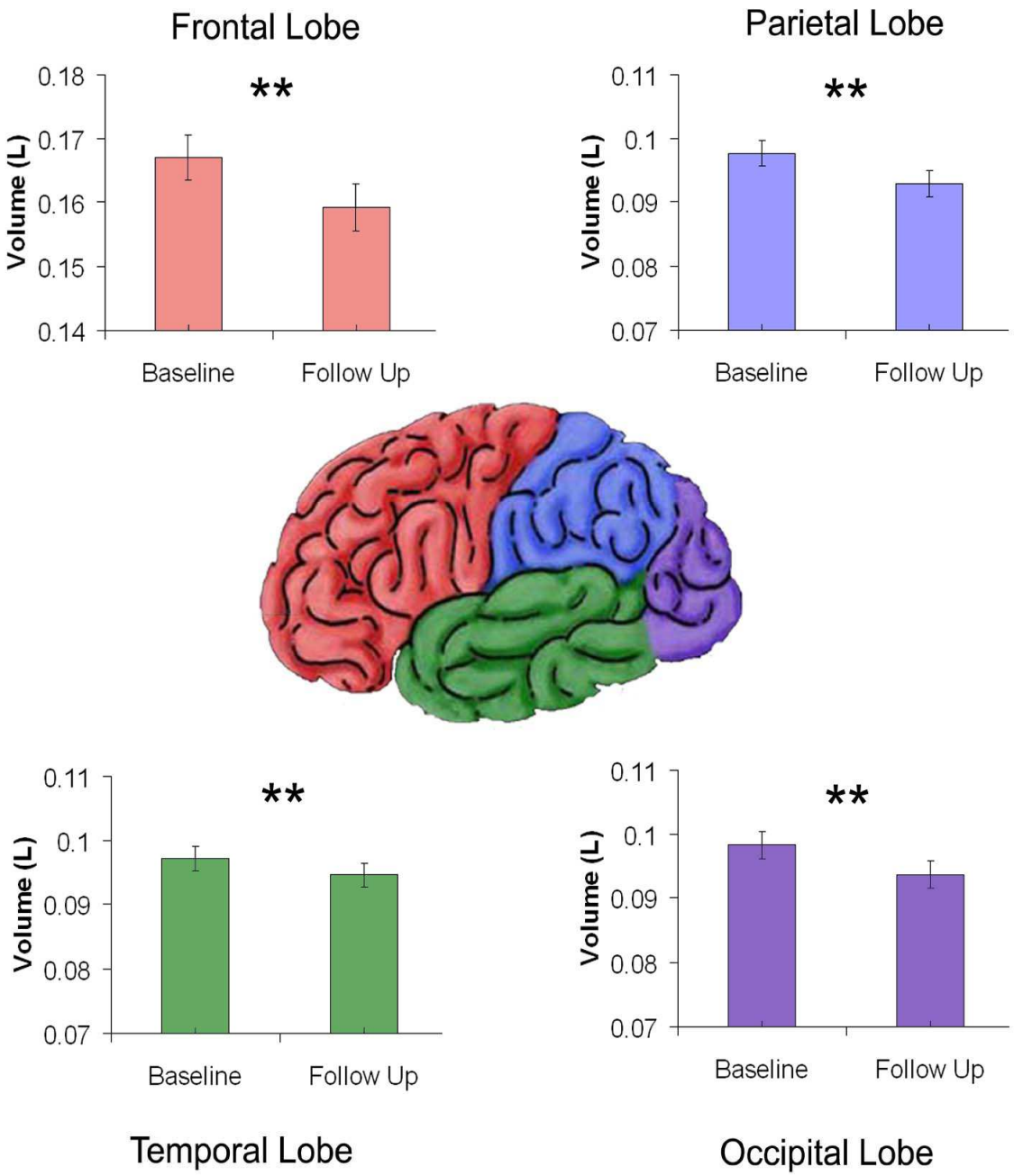

Figure 6-2: Mean grey matter volume in litres (± SEM) at baseline and follow-up for the frontal (pink), parietal (blue), occipital (purple) and temporal (green) grey matter ROIs for the 19 patients with first-episode schizophrenia **p<.001

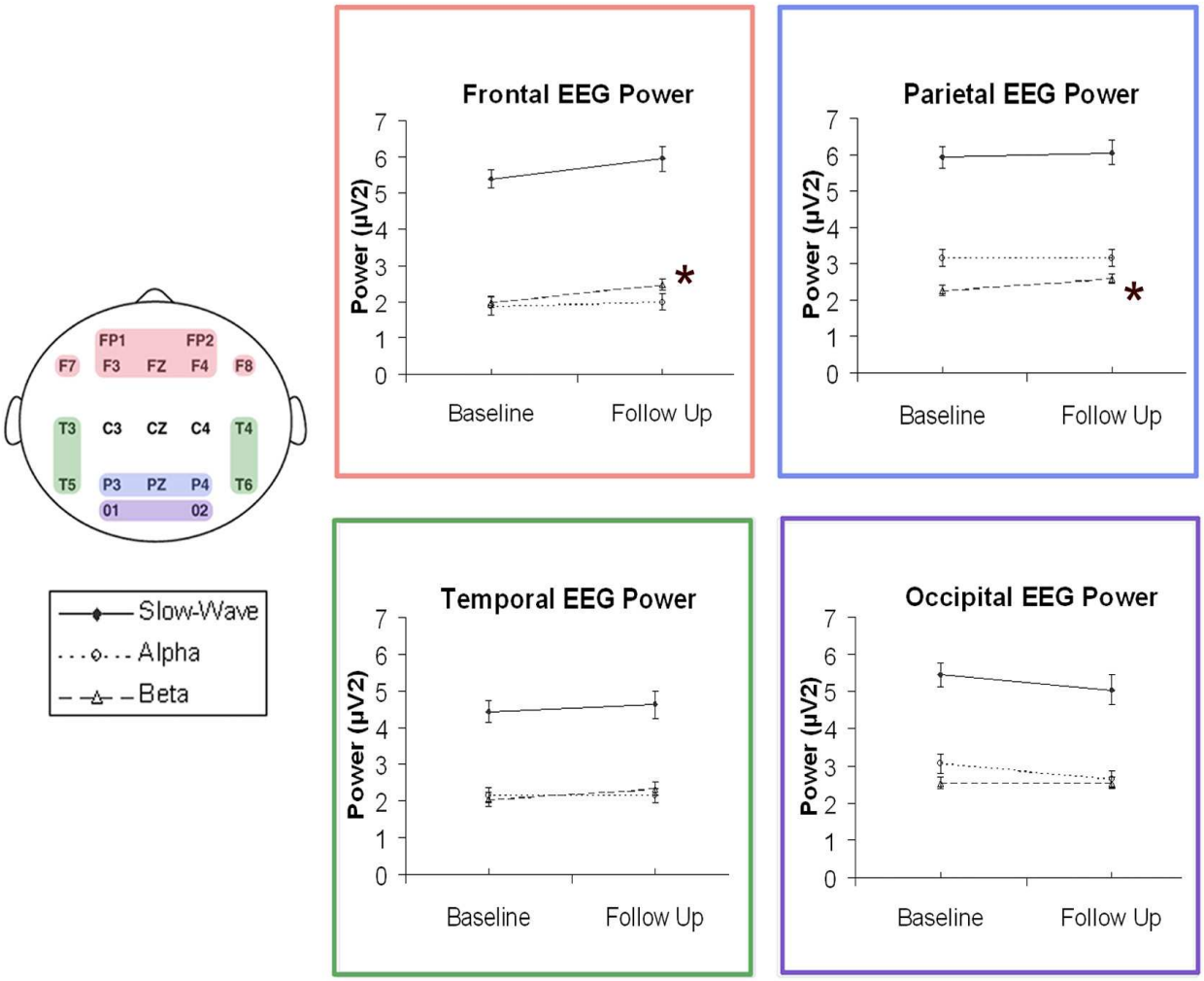

Figure 6-3: Mean absolute EEG power values in μV^2 (± SEM) at baseline and follow-up for the slow-wave (0.5-7.5Hz), alpha (8-12Hz) and beta (12.5-34.5Hz) frequency bands in the frontal, parietal, occipital and temporal 'EEG regions' for the 19 patients with first-episode schizophrenia *p<.05

6.6 DISCUSSION

In this study, structural MRI and resting EEG recordings were acquired from 19 patients with FES, both at baseline (within three months of first presentation to mental health services) and follow-up (2-3 years later). While GM volume was observed to decrease over the follow-up interval in the frontal, temporal, parietal and occipital lobes (Figure 6-2), absolute EEG power in the corresponding regions was preserved, or even increased over the same time period (Figure 6-3). This finding was consistent with the nearly significant negative correlations that were observed between the longitudinal GM changes in the frontal and parietal lobes and the longitudinal changes in EEG power in the corresponding cortical areas for the slow-wave frequency band.

These results point to a striking departure from the common trajectory of cortical anatomy and electrophysiology that I have previously reported in healthy participants over adolescence and early adulthood. In Chapter 2, a cross-sectional design was employed to examine the changes in GM volume and EEG power over adolescence in 138 healthy participants. A curvilinear decrease in frontal and parietal GM volume was observed that was mirrored by a curvilinear decrease in EEG power in corresponding regions, particularly for the slow-wave frequency band. To explain these findings I argued that EEG power could be considered to be a function of the number of active synapses (i.e. more synapses = higher amplitude = higher power) and the synchrony of the synaptic activity (i.e. higher synchrony = higher amplitude = higher power). Hence a reduction in GM volume (whether it be due to the elimination of entire neurons or

selected dendrites and axon terminals) would be associated with a reduction in the number of synapses and thus a reduction in EEG power, assuming that synchrony remained constant. In the study reported in this chapter, however, longitudinal reductions in EEG power were not observed to occur in 19 patients with FES, despite them exhibiting longitudinal reductions in GM volume across the brain. This result is consistent with a number of previous studies in the literature (Begic et al., 2000a; Omori et al., 1995). In actual fact, the FES patients in this study exhibited a trend for *increased* EEG power over the follow-up interval in the frontal, temporal and parietal 'EEG regions', especially in the slow-wave frequency band (Figure 6-3). This is in spite of the fact that the patients received neuroleptic medication and exhibited an improved clinical profile over the follow-up interval – events which have both previously been associated with reductions in slow-wave power (Harris et al., 1997; Saletu et al., 1994; Nagase, Okubo, & Toru, 1996; Begic, Hotujac, & Jokic-Begic, 2000b).

If the aforementioned model of EEG power holds true (i.e. power is a function of the number of synapses and the synchrony of activity), then this result indicates that the FES patients experienced an increase in neural synchrony over the first 2-3 years of illness. Although some previous studies have reported evidence of increased slow-wave and beta band power in patients with schizophrenia (Sponheim et al., 2000), there has been very little research investigating changes in synchrony at these frequencies. In fact, the only frequency band for which evidence of schizophrenia-related changes in neural synchrony has been well investigated is the gamma band (~40Hz). Interestingly, several previous studies have reported an abnormal *elevation* in gamma synchrony in patients with

schizophrenia, especially in patients with severe reality distortion (Baldeweg, Spence, Hirsch, & Gruzelier, 1998; Gordon et al., 2001) and disorganized thinking (Lee, Williams, Haig, & Gordon, 2003). If neural synchrony is mechanism by which the brain binds together discrete perceptual and/or cognitive stimuli, as has been previously suggested (Engel, Roelfsema, Fries, Brecht, & Singer, 1997), then it is possible to speculate that FES patients with abnormally high neural synchrony might bind together inappropriate thoughts or percepts, which could feasibly result in symptoms of disorganization and reality distortion (Lee, Williams, Breakspear, & Gordon, 2003; Silverstein, Kovacs, Corry, & Valone, 2000).

One possible confound relating to our finding of increased EEG power in the FES patients over the follow-up interval relates to the effects of their antipsychotic medication. There is some evidence to suggest that exposure to antipsychotic medication increases the likelihood of patients developing seizures (Hedges, Jeppson, & Whitehead, 2003). Given that seizures are ultimately a reflection of abnormally synchronous neural activity (the characteristically large amplitudes in the EEG during seizure activity being testament to this), it is possible that our observed increase in EEG power and hypothesized increase in EEG synchrony in the FES patients may in fact have resulted from their medication and not from the disease *per se*.

Whilst it would be practically and ethically impossible to deprive FES patients of antipsychotic medication for the first 2-3 years of their illness simply in order to address this potential confound, an alternative solution would be to longitudinally assess EEG

power and synchrony in non-psychotic psychiatric patients who were being described antipsychotic medications as part of their treatment program. For example, patients with eating disorders are occasionally prescribed atypical antipsychotic medications both to help them to gain weight, and to redress the dopaminergic and serotinergic imbalances that have been proposed occur in the disease (Bosanac, Norman, Burrows, & Beumont, 2005). If EEG power was found not to increase over the follow-up interval in these non-psychotic patients, then we would be confident in saying that the increased EEG power exhibited by the FES patients in this study was not merely a side-effect of their medication.

The results of this study (and any conclusions that are drawn from them) should be treated with caution until these findings are replicated, preferably by using a longitudinal design with a control sample and a larger patient sample. I am currently collecting data on phase synchrony in patients with FES for the slow-wave, alpha, beta and gamma frequency bands, in order to test the hypothesis regarding increased neural synchrony in patients with schizophrenia.

CHAPTER 7

GENERAL DISCUSSION

7.1 PREAMBLE

As outlined in Chapter 1, this thesis had four primary aims:

1. to identify and quantify the neuroanatomical changes associated with healthy adolescence and early adulthood,
2. to identify and quantify the neuroanatomical abnormalities present in patients with first-episode schizophrenia (FES), both at the time of their first presentation to mental health services with psychotic symptoms (baseline), and over the subsequent 2-3 years of illness (follow-up),
3. to identify and consider the origins of the relationship between FES patients' neuroanatomical abnormalities and their clinical profile at baseline, and,
4. to identify the relationship between longitudinal changes in neuroanatomy and longitudinal changes in electrophysiology in healthy participants and FES patients, and to consider the mechanisms underlying this relationship.

Empirical studies related to each of these objectives have been outlined in Chapters 2 to 6. This final chapter is comprised of four sections.

1. A reiteration of the main findings of the empirical studies, and their relevance to the primary aims of this thesis.
2. An integrated model of schizophrenia that related the empirical studies with the theoretical framework from the literature.

3. A discussion of the methodological limitations of the empirical studies.
4. A discussion of the ways in which these limitations could be addressed, and some suggestions for future research.

7.2 INTEGRATION OF THE EMPIRICAL FINDINGS

A number of theorists (e.g. Feinberg, 1982; Hoffman et al., 1989) have argued that schizophrenia arises from a dysfunction in the normative period of peripubescent brain maturation, in light of the fact that a) a number of studies have indicated that adolescence and early adulthood is a time of dramatic synaptic elimination in the healthy human brain (Huttenlocher, 1979; Rakic, Bourgeois, & Goldman-Rakic, 1994), and b) the peripubescent period is by far the most common age for schizophrenia to first present itself clinically. Thus the first aim of this thesis, addressed in Chapter 2, was to identify and quantify the structural brain changes that occur during adolescence and early adulthood in healthy individuals.

In Chapter 2, structural MRI scans were taken from 138 healthy participants between the ages of 10 and 30 years. Participants' brain scans were segmented into grey matter (GM) and white matter (WM) images, which were parcellated into four brain lobes before the aging-related changes in these lobes were inferred through the use of a regression model. This study demonstrated a significant reduction in frontal and parietal GM volume over 10 to 30 years (Figure 2-3). The reduction was non-linear, reflecting accelerated loss in the peripubescent period of approximately 10 to 20 years, relative to the later period between 20 and 30 years. By contrast, there was a significant increase in parietal WM

volume over 10 to 30 years of age (Figure 2-4). This increase was also non-linear, reflecting an accelerated gain in the corresponding peripubescent period[3].

These findings provide additional support for the view that the period around adolescence and early adulthood is a time of substantial change in the structure of the healthy human brain. I have argued that the opposing changes in GM and WM reflect the normative maturational processes of myelination and synaptic pruning respectively.

Given that previous studies have indicated that WM is constituted primarily of the myelinated axons of neuron bodies (Bear et al., 1996; Carlson, 2002; Kandel et al., 2000), the gain in peripubescent WM provides new evidence via a non-invasive, imaging methodology that myelination of the association cortices may indeed continue into the late twenties or early thirties (Yakovlev et al., 1967), and perhaps even later (Benes et al., 1994). The question of what molecular changes underlie the GM abnormalities characteristic of healthy aging has been investigated by a number of *in vitro, post mortem* studies. The majority of these studies indicate that the number of neurons in the human cerebrum remains more or less fixed from early childhood to old age (Purves, 1998; Williams et al., 1988). This suggests that the observed age-related GM changes in healthy adolescence primarily reflect a reduction in the volume of neuropil (i.e. dendrites, axon terminals and glial cells) rather than neuron death *per se*. This suggestion has important implications for the molecular underpinnings of the GM atrophy observed in the FES

[3] It is important to note that the curvilinear model (i.e. tissue volume vs log(age) was found to be a better predictor of tissue volume than the linear model (i.e. tissue volume vs age) for all four GM and all four WM regions, over the 10-30 year age bracket.

patients in Chapter 3, and I will draw on this suggestion in the integrated model of schizophrenia in Section 7.3.

The second, and in many ways central aim of this thesis was to identify and quantify the structural brain abnormalities present in patients with FES, both at the time of their first presentation to mental health services with psychotic symptoms (baseline) and over their first 2-3 years of illness (the follow-up interval). This topic was addressed in Chapters 3 and 4.

In Chapter 3, the analytical technique of voxel-based morphometry was used to identify, on a voxel-by-voxel basis, the regions where 41 patients with FES exhibited abnormally reduced or abnormally increased GM volumes at baseline, relative to 47 matched healthy controls. As shown in Figure 3-1, the FES patients exhibited widespread GM reductions at baseline relative to controls, particularly in the temporal and parietal cortices, right fronto-parietal cortex, left ventral prefrontal cortex and right cerebellum. In addition to these 'regions-of-reduction', the FES patients also exhibited a number of more circumscribed regions of GM increase at baseline, particularly in the occipital and somatosensory cortices and posterior cerebellum bilaterally (Figure 3-2). In addition to these GM abnormalities, the FES patients also exhibited several regions of WM abnormality at baseline, as reported in Chapter 4. Specifically, the FES patients exhibited volumetric WM reductions in the right frontal and left posterior temporal lobe (Figure 4-1), and volumetric increases in the fronto-parietal junction bilaterally (Figure 4-2). In summary, these results indicate that FES patients exhibit significant GM and WM

abnormalities, relative to age and sex matched healthy controls, at the time of their first presentation to mental health services with psychotic symptoms.

These results are supportive of many of the predictions made by the pre-eminent theories of schizophrenia discussed in Chapter 1, although given the sheer magnitude of the observed neuroanatomical abnormalities, this is perhaps not surprising. Firstly, the observation of widespread WM abnormalities in the FES patients at baseline is consistent with the model proposed by Bartzokis (2002), who argued that abnormal myelination could result in the symptoms of formal thought disorder, via an abnormality in the "*formation of associations between disparate events separated in time*" (p.674). Furthermore, the observation of structural abnormalities in the cerebellum in the FES patients at baseline is a notable finding that provides support for the predictions made by Andreasen (1999) in her theory of 'cognitive dysmetria'. The cerebellum has traditionally been thought to be exclusively responsible for the coordination of motor activities via its integration of complex sequences of muscle contractions (Bear et al., 1996). However, consistent with Andreasen's (1999) theory, recent research has consistently and robustly indicated that rather than being limited to the coordination of motor activities, the cerebellum also plays a role in the coordination of cognitive activities (Chen & Desmond, 2005; Gottwald, Wilde, Mihajlovic, & Mehdorn, 2004; Cabeza & Nyberg, 2000). The observation of structural abnormalities in the cerebellum of patients with FES provides a possible mechanism for the neural disintegration that has been so widely proposed as underlying the symptoms of schizophrenia (Andreasen, 1999; Friston, 1998; Frith, 1992). Further evidence for abnormalities in cortical/ cerebellar connectivity in schizophrenia

comes from a recent fMRI study by Whalley et al. (2005), who reported that subjects at high genetic risk of developing schizophrenia exhibited a decreased correlation between the blood-oxygen-level-dependant (BOLD) response of voxels recorded in the right medial PFC and those recorded in the contralateral cerebellum, compared to healthy controls. I will return to the concepts of dysfunctional connectivity and neural disintegration in a model of schizophrenia proposed in Section 7.3.

One theory not supported by the results of Chapter 3 was Weinberger's (1987) neurodevelopmental theory. Weinberger (1987) argued that schizophrenia ultimately resulted from a 'lesion' in the dorsolateral prefrontal cortex (DLPFC) which developed very early in life. Weinberger (1987) argued that this DLPFC lesion remained behaviourally silent until peripubescence, at which time it interacted with the normal brain maturation and increased cognitive load characteristic of this period and caused the onset of schizophrenia. While Weinberger (1987) was not specific as to the location of the DLPFC lesion, the most dorsal prefrontal region in which the FES patients were observed to exhibit baseline GM reductions in Chapter 3 was the middle frontal gyrus (Table 3-2). Thus these results do not support Weinberger's (1987) speculation as to the anatomical location of the proposed neurodevelopmental 'lesion' .

In addition to the structural brain abnormalities observed at baseline, the regions of increased longitudinal brain atrophy exhibited by 25 FES patients over the 2-3 year follow-up interval relative to 26 matched controls were identified using the analytical technique of tensor-based morphometry. The regions where the FES patients lost more

GM over the follow-up interval relative to the healthy controls were large and widespread, and encompassed much of the parietal and temporal cortices (Figure 3-3). The regions of increased longitudinal WM atrophy exhibited by the patients were, in comparison, considerably more circumscribed, and were confined to the inferior temporal lobe bilaterally (Figure 4-3). In contrast to the baseline results, there were no regions where the 26 control subjects were observed to lose more GM or WM over the follow-up interval compared to the 25 FES patients.

In summary, the longitudinal results indicate that patients with FES lose a greater volume of GM and WM over the first 2-3 years of illness, relative to healthy controls over the same time interval. I have speculated as to the mechanisms underlying these observed longitudinal abnormalities in Section 7-3. At first glance it may appear as though these results provide support for the 'neurodegenerative' theories of schizophrenia described in Chapter 1. However, as I will discuss further in Section 7.4, the methodology employed in these studies was not able to distinguish whether the abnormal, longitudinal tissue atrophy exhibited by the FES patients occurred smoothly and progressively over the 2-3 year follow-up interval, or whether it all occurred in a single, destructive period, and did not progress further after this point. Thus it was not possible, using this methodology, to determine whether schizophrenia is a true 'neurodegenerative' disease, in the sense of exhibiting progressive tissue atrophy over the course of the illness.

The third aim of this thesis, addressed in Chapter 5, was to identify the neuroanatomical correlates of FES patients' clinical profile at baseline, in order to better elucidate the

neuropathological origins of the symptoms of schizophrenia. To this end, four cortical regions were identified where 31 patients with FES exhibited volumetric GM reductions at baseline relative to 30 matched healthy controls (Table 5-1, Figure 5-1). The volumes of these four 'regions-of-reduction' were calculated for each of the FES patients, and correlated with their scores on three symptom dimensions: Disorganization, Reality Distortion and Psychomotor Poverty. Scores on these three symptom dimensions, which have been widely argued to encompass the fundamental factor structure of schizophrenic symptomatology (Andreasen et al., 1995; Liddle, 1987b), were calculated by summing items from the PANSS rating scale, following the study of Liddle et al. (1987a). The results obtained in Chapter 5 were somewhat paradoxical. It was observed that the higher the degree of Reality Distortion a patient was suffering from, the *less* grey matter atrophy, on average, that s/he exhibited in three of the four regions-of-reduction. In Section 5.5, I proposed three potential explanations for this curious result. One of these explanations (namely that severe GM atrophy precludes the formation of hallucinations or highly systematized delusions that would rate highly on the PANSS), stood out in particular as being consistent with several of the theories of schizophrenia proposed in the literature. I have incorporated this explanation into an integrated theory of schizophrenia proposed in Section 7.3.

The fourth aim of this thesis, which was addressed in Chapter 6, was to identify the relationship between longitudinal changes in brain structure and longitudinal changes in brain electrophysiology in patients with FES, and to compare it to the normative relationship between these two measures reported in Chapter 2. The rationale was that the

identification of an abnormal relationship between neuroanatomy and electrophysiology in patients with schizophrenia could potentially provide an insight into the functional consequences of the abnormal GM atrophy exhibited by the FES patients in Chapter 3, as well as the nature of the dysfunctional neural connectivity that has been so widely proposed to exist in schizophrenia.

In the study described in Chapter 2, a linear relationship was observed between the inferred age-related reductions in frontal and parietal GM volumes in healthy subjects, and the corresponding reductions in absolute EEG power averaged from the same cortical regions. A similar relationship, however, was not observed in Chapter 6, between the degree of longitudinal GM atrophy exhibited by 19 FES patients and the longitudinal changes in absolute EEG power from the corresponding cortical regions. Instead, while GM volume decreased significantly over the follow-up interval in each of the four cortical lobes in the FES patients (Figure 6-3), their absolute EEG power remained constant or even increased over the follow-up interval (Figure 6-4). In Section 6.6, I argued that an abnormal increase in neural synchrony could explain this irregular relationship. Furthermore, I argued that such an increase could provide the basis for the dysfunctional connectivity proposed in the literature as underlying the symptoms of schizophrenia. I have expanded upon this idea in an integrated model of schizophrenia presented in Section 7.3.

7.3 AN INTEGRATED MODEL OF SCHIZOPHRENIA: ASSIMILATING THE THEORETICAL FRAMEWORK AND EMPIRICAL OBSERVATIONS

The model presented in this section is an attempt to explain the aetiology, neuroanatomical properties and clinical characteristics of schizophrenia, by integrating the diverse range of empirical observations described in this thesis with the theoretical framework provided by the pre-eminent theories of schizophrenia from the literature. In proposing this model I have attempted to ensure the testability of each of its facets, so that they can be tested by future empirical investigations. The model, which is illustrated diagrammatically in Figure 7-1, begins by considering the mechanisms underlying the neuroanatomical abnormalities observed in the FES patients at baseline.

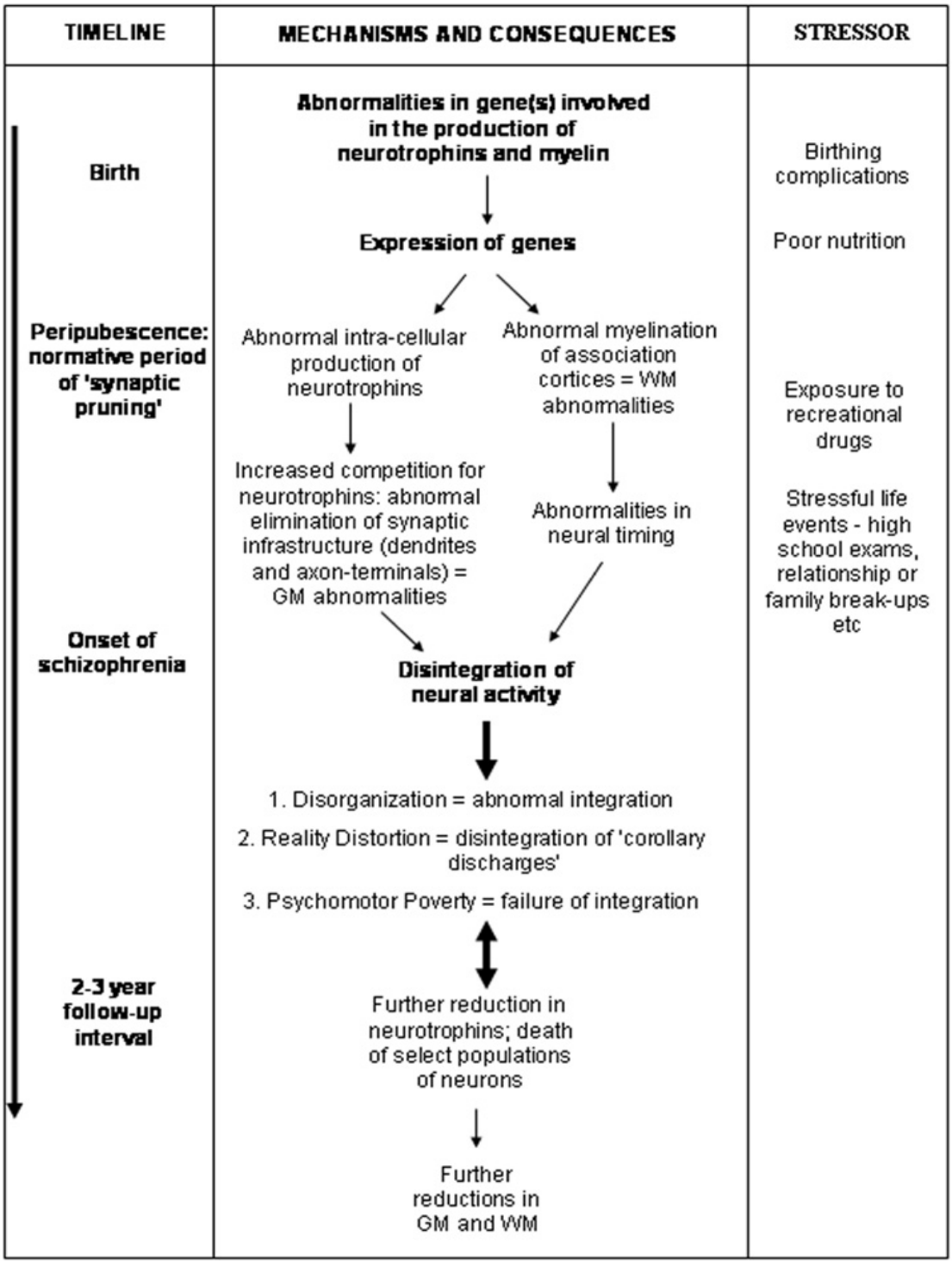

Figure 7-1: An integrated model of schizophrenia. In particular, the model focuses on the role of dysfunctional adolescent brain maturation in fermenting the neural disintegration that has been argued to underlie all of the symptoms of schizophrenia.

7.3.1 Mechanisms of neuroanatomical abnormality in FES patients at first clinical presentation

Adolescence and early adulthood is typically a time of enormous structural change in the healthy human brain. A number of previous studies have reported this period to be associated with accelerated GM loss, particularly in the frontal and parietal association cortices (Pfefferbaum et al., 1994; Sowell et al., 1999). These results are corroborated by the results of Chapter 2, which report a significant curvilinear decrease in frontal and parietal lobe GM in healthy participants between the ages of 10 and 30 years. Previous animal (Bourgeois et al., 1993) and human (Huttenlocher, 1979) studies have indicated that rather than being due to neuron death, this adolescent grey matter loss reflects the targeted elimination of synapses and their associated infrastructure (i.e. dendrites and axon terminals).

As previously discussed, there is evidence to suggest that this normative period of 'synaptic pruning' is triggered by a sudden reduction in the amount of available neurotrophins. This reduction in neurotrophins has been thought to be triggered by the enormous hormonal changes associated with puberty (Feinberg, 1982). According to Purves (1998), a synapse (or rather its associated infrastructure) must receive a certain critical amount of neurotrophin from the post-synaptic cell in order to survive. A failure to receive this critical amount of neurotrophin will result in the elimination of the synaptic infrastructure.

It is suggested that it is this reduction in synaptic infrastructure that underlies the GM loss characteristic of healthy adolescence, rather than the elimination of neuron bodies. This proposal is consistent with the prevailing theories of aging, which argue that the number of neurons in the human cerebrum remains relatively stable from early childhood until late adulthood, after which time there is a progressive reduction in neuron number, possibly resulting from the onset of dementia-type illnesses typically associated with old age (Williams et al., 1988).[4]

Given the sheer magnitude of this 'synaptic prune' (Bourgeois and Rakic (1993), for example, estimated that 5000 synapses were being eliminated per minute in the visual cortex alone in macaque monkeys undergoing puberty), it seems reasonable to assume that a relatively minor abnormality in this process could have major implications for cerebral structure and function. Consistent with this assumption, Feinberg (1982) proposed the idea of schizophrenia arising because of an abnormality in this process of targeted synaptic elimination, such that "*too many, too few or the wrong synapses are eliminated*" (p.331). Support for Feinberg's (1982) theory comes from the fact that while patients with schizophrenia have been observed to have a similar total number of neurons compared to controls (Pakkenberg, 1993), they show an increased neuronal density (i.e. an increased number of neurons per unit of cortical volume; Selemon, Rajkowska, & Goldman-Rakic, 1998), which is consistent with a reduction in cortical volume resulting from a loss of synaptic infrastructure (Selemon et al., 1999).

[4] As a caveat to this, Purves (1998) has argued that if a neuron fails to obtain a certain critical amount of neurotrophin from all its synapses combined, as frequently occurs in the developing neonatal brain, then the neuron itself will die, probably through apoptosis. I will return to this point later in this section.

Hence there is circumstantial evidence supporting the hypothesis that schizophrenia may result from an abnormality in the dramatic period of synaptic pruning typically associated with healthy adolescence. In Chapter 4, I suggested that this abnormality in synaptic pruning most likely occurs in the months prior to the onset of psychosis, although a recent study by Pantelis et al. (2003) has indicated that neuroanatomical abnormalities may precede the onset of psychosis by longer than this. I subsequently argued that this dysfunction in synaptic pruning and the corresponding loss of synaptic infrastructure is primarily responsible for the widespread GM abnormalities exhibited by the FES patients at baseline.

In addition to the widespread GM abnormalities observed at baseline in Chapter 3, there were also a number of regions of WM abnormality observed in Chapter 4. The fact that these WM abnormalities did not, for the most part, correspond to the regions of GM abnormality (with a possible exception in the right frontal lobe; see Tables 3-1, 3-2, 4-1 and 4-2) suggests that the WM abnormalities arose because of a process independent of the dysfunctional synaptic pruning that I argue to underlie the GM abnormalities. Consistent with this suggestion, several lines of evidence have indicated that abnormalities in axonal myelination are present in patients with schizophrenia, and thus may underlie the WM abnormalities reported in Chapter 4. For example, patients with schizophrenia have been observed to show WM hyperintensities (Sachdev et al., 1999) and decreased magnetization transfer ratios (Foong et al., 2000) on MR images, as well as reductions in fractional anisotropy in diffusion tensor images (Kubicki et al., 2005), all of which have been associated with impaired myelination. Furthermore, a recent

immunohistochemical study has reported abnormally reduced numbers of oligodendrocytes (glial cells involved in the production and maintenance of myelin) in the WM of the superior frontal gyrus in patients with schizophrenia *post mortem* (Hof et al., 2003). Finally, a recent genetic study using DNA microarray analysis has reported five genes implicated in the formation and maintenance of myelin sheaths to be down-regulated in patients with schizophrenia relative to healthy controls (Hakak et al., 2001). Thus while there is evidence indicating that abnormalities in synaptic pruning underlie the GM abnormalities at baseline, there is also evidence indicating that an independent irregularity in axonal myelination underlies the observed WM abnormalities.

7.3.2 Neuroanatomical abnormalities and schizophrenic symptomatology

In Section 7.3.1, I argued that the GM abnormalities observed in the FES patients at baseline were due primarily to a reduction in synaptic infrastructure (i.e. dendrites and axon terminals), caused by a dysfunction in the normative period of adolescent brain maturation. In contrast to this, I argued that the baseline WM abnormalities primarily resulted from an independent dysfunction in the processes of axonal myelination. The purpose of this current section is to consider the mechanism by which these neuroanatomical abnormalities result in the symptoms of schizophrenia.

It seems reasonable to assume that an abnormality in the normative process of synaptic pruning during adolescence would result in:

1. The elimination of synapses that would normally have been preserved – reflected in the regions of GM reduction at baseline in the FES patients.
2. The preservation of synapses that would normally have been eliminated – reflected in the regions of GM increase at baseline in the FES patients.

It is easy to imagine how such an abnormality in synaptic pruning could cause disturbances in inter-cellular communication, and a subsequent disintegration of neural activity. Such a disintegration of neural activity would likely be exacerbated by concurrent abnormalities in axonal myelination, given the role myelin plays in the regulation of neural timing, via its role in modulating the conduction velocities of action potentials along axons.

It is possible that a clinical consequence of this disintegrated neural activity would be the formation of abnormal associations between disparate sensations and cognitions, which is the characteristic feature of Disorganization. And in line with Frith's (1992) theory (Section 1.5.6), such a disintegration of neural activity could also underlie the symptoms of Reality Distortion, if it resulted in an uncoupling of the neural discharges instigating willed actions (such as movement, thought and speech) and the 'corollary discharges' underlying patients' awareness of their intention to act.

I have suggested, however, that the formation of abnormal sensory and cognitive associations could only occur up to a point in the schizophrenic brain. That is, above a certain level of neural disintegration, the brain would simply be unable to bind together

even inappropriate thoughts or perceptions, such as has been proposed to underlie the symptoms of Disorganization and Reality Distortion. In this event, the brain might simply 'shut down', "*much as a computer locks up when it cannot match signals sent at an incorrect rate or to an incorrect place*" in the words of Nancy Andreasen (Andreasen, 1999, p.785).

Such an occurrence would be expected to be associated with:

1) a decrease in patients' level of Disorganization and Reality Distortion, and,
2) an increase in their levels of Psychomotor Poverty.

The first prediction is supported by the results of Chapter 5, in which patients with severe Reality Distortion exhibited comparatively less GM atrophy than their counterparts who were not suffering from severe Reality Distortion. And although a significant correlation between patients' levels of Psychomotor Poverty and their degree of GM atrophy was not observed in Chapter 5, a number of previous studies have reported this association in patients with schizophrenia (Baare et al., 1999; Chua et al., 1997; Gur et al., 2000). Furthermore, if there was found to be progressive brain atrophy over the course of schizophrenia (a possibility that has some (Cahn et al., 2002b) if not unanimous (DeLisi et al., 2005) support), then it would be predicted that patients with a long duration of illness would suffer from increased levels of Psychomotor Poverty. The fact that chronically ill patients have been shown consistently to exhibit a more 'negative' clinical profile than patients with FES (McGlashan, 1998) adds further support to the hypothesis.

7.3.3 Progressive brain atrophy in first-episode schizophrenia

In Section 7.3.2, I discussed the mechanisms underlying the structural brain abnormalities exhibited by the FES patients at baseline. The purpose of this section is to examine the mechanisms underlying the abnormal degree of longitudinal brain atrophy exhibited by the FES patients over the 2-3 year follow-up interval.

In the study described in Chapter 3, FES patients were observed to lose a significantly greater volume of GM over the first 2-3 years of illness than did a matched group of healthy control subjects over the same interval. The regions affected by this abnormal, longitudinal GM atrophy were widespread, and encompassed much of the parietal and temporal lobes (Figure 3-3). An interesting pattern emerged, however, when these regions of longitudinal GM atrophy were compared to the regions of longitudinal WM atrophy. While the regions of longitudinal WM atrophy were far more circumscribed than the regions of GM atrophy, and occupied only a small portion of the temporal lobe (Figure 4-3), they were observed to occur immediately medial to the largest and most statistically significant region of longitudinal GM loss, that is, in the temporal and parietal lobes bilaterally (Figure 4-4). The spatial correspondence of these regions of longitudinal grey and white matter atrophy suggests that they may be related.

In Section 7.3.1, I argued that the GM atrophy evident in the FES patients at baseline primarily reflected the elimination of synaptic infrastructure, resulting from a

pathological reduction in the amount of available neurotrophins. However, as previously discussed, there is evidence to suggest that if a neuron fails to obtain a certain critical amount of neurotrophin from all its synapses combined, then the neuron itself (i.e. not merely its synaptic infrastructure) will die (Purves, 1998), probably through apoptosis (Henderson, 1996).

Therefore, I suggest that while the majority of the GM atrophy observed over the follow-up interval in the FES patients was due to the elimination of synaptic infrastructure resulting from relatively mild shortages in the amount of available neurotrophin, the development of a critical shortage in the amount of neurotrophin available to the temporal lobe neurons some time over the follow-up interval resulted in the death of these neurons. The death of these cells would result in a reduction in both GM volume and WM volume, as the myelinated axons of the neurons would be dismantled, and consumed by phagocytes (Kandel et al., 2000). The fact that the majority of cortical neurons are pyramidal neurons (Braitenberg, 1991), which project their axons perpendicular to the surface of the cortex (i.e. directly medial for temporal lobe neurons) means that the longitudinal GM reductions in the temporal lobe exhibited by the FES patients in Chapter 3 would be expected to result in WM reductions similar to those observed in Chapter 4 if the GM reductions were due to the elimination of these neurons.

In summary, I suggest that a peripubescent reduction of neurotrophins results in the elimination of synaptic infrastructure in the FES patients, and that this accounts for the majority of the GM abnormalities observed at baseline and over the follow-up interval.

Further neurotrophin reductions to below a critical level in certain temporal lobe neurons would result in the death of these neurons and the elimination of their myelinated axons.

7.3.4 The relationship between neuroanatomy and electrophysiology in patients with FES

In Chapter 2, I explored the relationship between changes in cortical GM and changes in absolute EEG power in healthy adolescents/ young adults. I observed a strong linear relationship between the two measures, that is, as GM volume decreased so did absolute EEG power in the corresponding cortical regions. To explain this result I proposed a basic model of absolute EEG power which argued that EEG power could be considered a function of:

1) the number of active cortical synapses – with more synapses resulting in higher EEG amplitudes and therefore higher power, and
2) the synchronicity of the synaptic activity – with higher synchrony resulting in higher EEG amplitudes and therefore higher power.

In Chapter 6, however, the longitudinal GM reductions experienced by the FES patients were not observed to be associated with a corresponding decrease in EEG power in the corresponding cortical regions. Instead, absolute EEG power remained constant or even increased (in the case of the frontal and parietal lobe beta power (Figure 6-4)) in these patients over the follow-up interval. Regardless of whether the longitudinal GM atrophy

exhibited by the FES patients resulted from the elimination of neurons, dendrites, axon terminals or a combination of them all, it would nonetheless be associated with a reduction in the number of cortical synapses. Hence if the basic model of EEG power proposed above is correct, then a reduction in the number of cortical synapses in the FES patients, combined with a preservation of their absolute EEG power, would indicate an *elevation* in the synchronicity of the synaptic activity in these patients. In light of the increasingly popular theories which argue that neural synchrony is the mechanism by which the brain binds together sensory and/or cognitive stimuli (Engel et al., 1997; Milner, 1974; Bressler, 1995), it is possible to speculate that FES patients with abnormally elevated neural synchrony could bind together inappropriate thoughts or perceptions, which could underlie the symptoms of Disorganization, Reality Distortion and, ultimately, Psychomotor Poverty (Section 7.3.2).

Thus, I suggest that people who go on to develop schizophrenia exhibit abnormalities in synaptic pruning and axonal myelination in adolescence. These neuroanatomical irregularities ultimately result in an abnormal elevation in the synchronicity of their neural activity. This abnormally elevated neural synchrony leads to the symptoms of schizophrenia, via the mechanisms of neural disintegration discussed in Section 7.3.2.

7.4 LIMITATIONS OF THE THESIS

While attempting to be as thorough and rigorous as possible with respect to the methodologies employed for subject recruitment, data acquisition and data analysis, there are three main methodological limitations of this thesis. Addressing these limitations in future research would be of great value in confirming the validity of the empirical observations made in this thesis and validating the various facets of the model proposed in Section 7.3.

The first, and I would argue most severe, limitation of this thesis relates to the fact that the longitudinal brain atrophy exhibited by the FES patients in Chapters 3 and 4 was inferred on the basis of only two MRI scans. By acquiring only two MRI scans, it was impossible to determine exactly when, in the follow-up interval, the longitudinal brain atrophy actually occurred. Furthermore, it was also impossible to determine whether the longitudinal brain atrophy occurred in a single, destructive period and did not progress further beyond this point, or whether it progressed gradually and smoothly over the follow-up interval. Furthermore, it was also impossible to determine whether the longitudinal brain atrophy would have continued to progress after the follow-up scan. The upshot of this methodological limitation is that it was not possible to determine whether schizophrenia is a ‘neurodegenerative’ disease in the sense of exhibiting a progressive deterioration in brain structure.

Consider, for example, the possibility that the GM abnormalities exhibited by the FES patients at baseline was, in fact, caused by a dysfunction in the normative period of synaptic pruning during adolescence. Given that this period of synaptic pruning only lasts for a couple of years in healthy people, it seems reasonable to assume that the GM abnormalities associated with a dysfunction in this process would only continue to progress for a couple of years also. If, for example, the GM abnormalities began to present approximately 12 months prior to the onset of psychosis in the FES patients (as is consistent with the results of Pantelis et al., 2003), then this would mean that in this scenario, the longitudinal GM atrophy would have occurred in its entirety after, say, one year of the 2-3 year follow-up interval.

Compare this to the contrasting scenario in which the longitudinal GM atrophy exhibited by the FES patients resulted from a gradual, progressive process of neurodegeneration over the entire follow-up interval, and quite possibly beyond. Using the methodology employed in this study, it was impossible to distinguish between the validity of these two scenarios. Furthermore, while the acquisition of one or more additional MRI scans within the 2-3 year follow-up interval (e.g. a scan at baseline, and a scan at 1 year post, 2 years post and 3 years post baseline) would be an obvious way to overcome this issue, it would not provide any insight into the progression (or lack thereof) of structural brain abnormality over the course of the disease, which can be 50 or more years for many patients. In order to investigate this more general issue, and hence elucidate whether or not schizophrenia is truly a ‘neurodegenerative’ disease, it would be necessary to acquire MRI scans regularly over the course of the illness. And while extending the period of

longitudinal investigation beyond 2-3 years was obviously not possible in the context of a PhD thesis, it would be an enormously valuable task for future research, as it would provide an unparalleled insight into the nature and course of the neuroanatomical abnormalities that underpin schizophrenia.

The second limitation of this thesis relates to the size of subject samples investigated in Chapters 3 to 6. The sample sizes for these studies were limited by the difficulties inherent in recruiting patients within three months of their first presentation to mental health services with psychotic symptoms. Nevertheless, the sample sizes of between 30 and 40 for baseline studies and between 20 and 25 for the longitudinal studies were close to the minimum size recommended to ensure robust statistical analyses for studies of this sort (Aron & Aron, 1994).

The results of studies with small sample sizes dominate the imaging literature in psychiatry, and may account for some of the numerous conflicting findings. In general, all things being equal, far more confidence can be placed on the results of studies with large sample sizes (Gordon, Cooper, Rennie, Hermens, & Williams, 2005). Replicating the results of this thesis in future studies with larger subject numbers would provide convincing evidence as to the validity of the results, and would add weight to the conclusions that were drawn from them.

The third limitation of this thesis relates to its failure to include an index of neural synchrony in the analysis of the EEG data. In Section 7.3, I suggested that a

disintegration of neural activity, resulting from abnormalities in synaptic pruning, and the myelin-regulated timing of neural activity, was ultimately responsible for the symptoms of schizophrenia. I also argued that a result of this disintegrated neural activity was an abnormal *increase* in the synchrony of the neural activity exhibited by FES patients. In light of the increasingly popular theory which argues for neural synchrony as being the mechanism by which the brain binds together discrete perceptual and/or cognitive stimuli (Engel et al., 1997; Milner, 1974; Bressler, 1995), I speculated that FES patients with abnormally high levels of neural synchrony might bind together inappropriate thoughts or perceptions, which could feasibly result in Disorganization, Reality Distortion and, ultimately, Psychomotor Poverty.

This hypothesis, however, is highly speculative and would require the direct observation of increased neural synchrony in FES patients before it would be possible to be confident as to its validity. Calculating the synchrony of EEG data is a complex and specialized task, and one that only a handful of laboratories around the world have the capacity to accomplish. Furthermore, the vast majority of previous studies into neural synchrony in schizophrenia have focused on the gamma (approximately 40Hz) frequency band, while I have specifically predicted increased synchrony in the lower frequency bands (i.e. alpha, beta and slow-wave). Investigating the empirical evidence for increased neural synchrony in all the EEG frequency bands in patients with FES would, I believe, be an enormously fertile area for future research, and would enable a deeper understanding of the mechanisms underlying the functional brain abnormalities characteristic of schizophrenia.

7.5 RELATED ISSUES AND FUTURE RESEARCH

I suggest that the first goal of any future research program stemming from this thesis should be to address the methodological limitations discussed in Section 7.4. In spite of this, however, there were a number of other related issues that could potentially provide a fertile ground for future empirical investigations.

Given that this thesis investigated the clinical and electrophysiological correlates of the neuroanatomical abnormalities observed in patients with FES, one issue begging further investigation is the *neuropsychological* correlates of the observed structural brain abnormalities. It has been well documented that schizophrenia is associated with a cognitive decline over the first few years of illness (Saykin et al., 1994), particularly in terms of executive functioning, memory, attention and theory-of-mind. This cognitive decline is a particularly destructive feature of the disease, which is responsible for some of its most debilitating and stigmatising effects such as the difficulties typically experienced by patients in obtaining and maintaining steady employment. The nature of the relationship between the progressive structural neuropathology and cognitive decline exhibited by patients with FES remains poorly understood, with only a few studies reported in the literature (e.g. Ho et al., 2003; Hoff et al., 1999) Elucidating the nature of this relationship could potentially provide useful information as to how the cognitive deficits typical of FES could be targeted pharmacologically.

The relationship between longitudinal neuropathology and cognitive decline was investigated in a study of which I was a major contributor (see Appendix 1). In this study, we calculated the volumes of the frontal and temporal lobes at baseline and at 2-3 year follow-up, for 20 patients with FES. Using a similar masking methodology to the one employed in Chapters 2, 5 and 6 of this thesis, the volumes of these two lobes were calculated by automatically applying manually-drawn masks in MNI space to patients' spatially normalized brain images. We investigated the relationship between progressive brain atrophy and progressive cognitive decline by correlating patients' longitudinal changes in frontal and temporal lobe volume with their changes on a comprehensive battery of neuropsychological tests that was designed to assess a wide range of cognitive functions.

While we observed a significant reduction in FES patients' frontal lobes volumes, visual memory scores and estimated IQ over the follow-up interval, we also observed a significant correlation between patients' longitudinal reductions in frontal lobe volume and their longitudinal reductions in verbal learning and memory scores. The results of this study suggest that verbal learning and memory may be particularly sensitive indices of frontal lobe atrophy in patients with FES. In spite of this, however, the structural underpinnings of the other cognitive deficits associated with schizophrenia remained elusive in this study, and might be better investigated through the use of high-resolution functional imaging techniques such as functional MRI (fMRI) and magnetoencephelography (MEG).

A second unaddressed issue relevant to the findings of this thesis relates to the specificity of the observed neuroanatomical abnormalities in the FES patients. This issue has important theoretical implications for the distinction that the discipline of psychiatry has traditionally made between the diseases of schizophrenia and the other major psychotic illness of bipolar disorder (for example, see DSM-IV; American Psychiatric Association, 1994). Tim Crow (1995) has argued that in spite of their dissimilar clinical presentations (with the characteristic oscillations between mania and depression in bipolar disorder being the most obvious example), schizophrenia and bipolar disorder should not be considered as distinct diseases, but rather as simply different manifestations of a single pathological entity of 'psychosis'. In particular, Crow (1995) has argued that these 'two' diseases share the same aetiologies and, importantly in the context of this thesis, exhibit a "*homogeneity of structural brain changes*" (p.139). Crow's (1995) 'unified psychosis theory', if correct, would have important implications for psychiatry's conceptualisations of schizophrenia and bipolar disorder, and the treatment protocols that are used to manage them. In the context of this thesis, Crow's (1995) theory would imply that the model proposed in Section 7.3 to explain the nature and aetiology of schizophrenia would also be applicable to bipolar disorder.

The specific issue of whether the neuroanatomical abnormalities reported in this thesis were specific to patients with FES, or whether they also occurred in patients with first-episode bipolar disorder (FEBD) was investigated in a study of which, again, I was a major contributor (see Appendix 2, and Farrow et al. (2005)). In this study, we used voxel-based morphometry to assess the regions in which 22 patients with FES and 8

patients with FEBD exhibited differences in grey and white matter volumes, both at baseline and over a 2-year follow-up interval. The results of the study clearly indicated that the FES and FEBD patients exhibited markedly different neuroanatomical substrates, with the FES patients exhibiting reduced GM at baseline in the inferior frontal cortex, and experiencing a greater degree of longitudinal GM atrophy over the follow-up interval in large proportion of the temporal and parietal cortices, relative to the FEBD patients. Thus rather than indicating a "*homogeneity of structural brain changes*", as postulated by Crow (1995), the results of this study suggest that patients with FES exhibit, for the most part, more severe neuroanatomical abnormalities relative to patients with FEBD, both at the time of their presentation to mental health services and over the first two years of illness.

There is one final issue that was not addressed directly in the thesis, but is highly relevant to the general conclusions that I have drawn. It relates to the genetic underpinnings of schizophrenia. As discussed in Section 1.5.6, schizophrenia undoubtedly has a strong genetic component, with previous studies indicating that the monozygotic twins of schizophrenic patients have a greater than 40% chance of developing the disease themselves, at a rate 40 times higher than the general population (Gottesman et al., 1987). While no single gene has been identified as being reliably abnormal in patients with schizophrenia, a number of genes have been implicated including COMT (Egan et al., 2001) and DISC1 (Zhang et al., 2006). However, on the basis of the model of schizophrenia proposed in Section 7.3, there may be reason to suspect that abnormalities

in genes involved in the production and regulation of neurotrophins might play a role in the aetiology of schizophrenia.

In Section 7.3, it was suggested that schizophrenia results from an abnormality in the normative period of peripubescent synaptic pruning. Furthermore, it was argued that this abnormality in synaptic pruning was ultimately caused by the expression, in adolescence, of a gene (or genes) involved in the intra-cellular production of some type (or types) of neurotrophin, combined with an exposure to a precipitating diathesis. Attempting to identify this abnormal gene (or genes) would, I believe, be a fertile area for future research, as it would have the potential to elucidate the origins of schizophrenia at the most fundamental level. Of the ten or more trophic factors that are thought to be produced in the human brain, there is only a small subset for which the candidate genes are known. For example, the gene coding for BDNF has been located at chromosome 11p13 in humans, while the gene coding for NGF has been located at chromosome 1p21-p22.1 (Shoval & Weizman, 2005). Of the few previous studies that have looked for evidence of abnormal polymorphisms in genes coding for neurotrophins in patients, at least two have reported results that are consistent with the aforementioned hypothesis. For example, while Neves-Pereira et al. (2005) reported patients with schizophrenia to exhibit an abnormally increased tendency to exhibit the valine (as opposed to methionine) amino acid at codon 66 of the BDNF gene, Szekeres et al. (2003) reported that a single nucleotide substitution (cytosine to thymine) at codon 270 in the BDNF gene was associated with an increased susceptibility for schizophrenia. Hence there is already some evidence suggesting that certain polymorphisms of the BDNF gene are a predisposing

factor for the development of schizophrenia, possibly via a mechanism such as that described in Section 7.3. Concretely identifying the genetic precursors of schizophrenia would raise the possibility of ultimately being able to prevent subjects with a high genetic risk for schizophrenia from developing the disease, by shielding them from some of the environmental stressors that have been argued to precipitate the onset of schizophrenia.

7.6 CONCLUDING COMMENTS

Schizophrenia is one of the most devastating diseases to affect humanity. Understanding the origins and nature of schizophrenia is one of the most important challenges facing modern medical science, as it underpins the extent to which the disease can be successfully treated. To this end, this thesis has attempted to elucidate the neuroanatomical abnormalities exhibited by patients suffering from their first episode of schizophrenia, both at the time of their first presentation to mental health services, and over their first few years of illness. Furthermore, this thesis has also attempted to elucidate the clinical and electrophysiological correlates of these neuroanatomical abnormalities, and to provide a speculative model of the origins and nature of schizophrenia, based on an integration of the theoretical framework provided in the literature, and the empirical observations made in this thesis and elsewhere. My hope is that the ideas and observations presented here will go some way towards solving the tragic riddle that is schizophrenia, and provide some basis for future research into the disease.

REFERENCES

Abi-Dargham, A., Rodenhiser, J., Printz, D., Zea-Ponce, Y., Gil, R., Kegeles, L. S. et al. (2000). Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America, 97,* 8104-8109.

Aboitiz, F., Scheibel, A. B., Fisher, R. S., & Zaidel, E. (1992). Fiber composition of the human corpus callosum. *Brain Research, 598,* 143-153.

Akil, M., Pierri, J. N., Whitehead, R. E., Edgar, C. L., Mohila, C., Sampson, A. R. et al. (1999). Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *American Journal of Psychiatry, 156,* 1580-1589.

American Psychiatric Association (1994). *Diagnostic and statistical manual of mental disorders, revised 4th edition*. Washington D.C: American Psychiatric Association.

Ananth, H., Popescu, I., Critchley, H. D., Good, C. D., Frackowiak, R. S., & Dolan, R. J. (2002). Cortical and subcortical gray matter abnormalities in schizophrenia determined through structural magnetic resonance imaging with optimized volumetric voxel-based morphometry. *American Journal of Psychiatry, 159,* 1497-1505.

Andreasen, N. C. (1979). Thought, language and communication disorders: 2. Diagnostic significance. *Archives of General Psychiatry, 36,* 1325-1330.

Andreasen, N. C. (1984a). *Scale for the Assessment of Negative Symptoms (SANS).* Iowa City: University of Iowa.

Andreasen, N. C. (1984b). *Scale for the Assessment of Positive Symptoms (SAPS).* Iowa City: University of Iowa.

Andreasen, N. C. (1999). A unitary model of schizophrenia: Bleuler's "fragmented phrene" as schizencephaly. *Archives of General Psychiatry, 56,* 781-787.

Andreasen, N. C., Arndt, S., Alliger, R., Miller, D., & Flaum, M. (1995). Symptoms of schizophrenia. Methods, meanings, and mechanisms. *Archives of General Psychiatry, 52,* 341-351.

Andreasen, N. C., Ehrhardt, J. C., Swayze, V. W., Alliger, R. J., Yuh, W. T., Cohen, G. et al. (1990). Magnetic resonance imaging of the brain in schizophrenia. The

pathophysiologic significance of structural abnormalities. *Archives of General Psychiatry, 47,* 35-44.

Andreasen, N. C., Nopoulos, P., O'Leary, D. S., Miller, D. D., Wassink, T., & Flaum, M. (1999). Defining the phenotype of schizophrenia: cognitive dysmetria and its neural mechanisms. *Biological Psychiatry, 46,* 908-920.

Arndt, S., Tyrrell, G., Flaum, M., & Andreasen, N. C. (1992). Comorbidity of substance abuse and schizophrenia: the role of pre-morbid adjustment. *Psychological Medicine, 22,* 379-388.

Aron, A. & Aron, E. (1994). *Statistics for Psychology*. New Jersey: Prentice-Hall.

Ashburner, J., Andersson, J. L., & Friston, K. J. (2000). Image registration using a symmetric prior--in three dimensions. *Human Brain Mapping, 9,* 212-225.

Ashburner, J. & Friston, K. (1999). Nonlinear spatial normalization using basis functions. *Human Brain Mapping, 7,* 254-266.

Ashburner, J. & Friston, K. (2004). Morphometry. In R.S.Frackowiak, J. Ashburner, K. Friston, & W. Penny (Eds.), *Human brain function* (pp. 1-21). Amsterdam, Boston: Elsevier Academic Press.

Ashburner, J. & Friston, K. J. (2000). Voxel-based morphometry - the methods. *NeuroImage, 11,* 805-821.

Ashburner, J. & Friston, K. J. (2001). Why voxel-based morphometry should be used. *NeuroImage, 14,* 1238-43.

Ashe, P. C., Berry, M. D., & Boulton, A. A. (2001). Schizophrenia, a neurodegenerative disorder with neurodevelopmental antecedents. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 25,* 691-707.

Baare, W. FC., Hulshoff Pol, H. E., Hijman, R., Mali, W. P. T., Viergever, M. A., & Kahn, R. S. (1999). Volumetric analysis of frontal lobe regions in schizophrenia: relation to cognitive function and sympomatology. *Biological Psychiatry, 45,* 1597-1605.

Baddeley, A., Emslie, H., & Nimmo-Smith, I. (1993). The Spot-the-Word test: a robust estimate of verbal intelligence based on lexical decision. *British Journal of Clinical Psychology, 32,* 55-65.

Bagary, M. S., Symms, M. R., Barker, G. J., Mutsatsa, S. H., Joyce, E. M., & Ron, M. A. (2003). Gray and white matter brain abnormalities in first-episode schizophrenia inferred from magnetization transfer imaging. *Archives of General Psychiatry, 60,* 779-788.

Baldeweg, T., Spence, S., Hirsch, S. R., & Gruzelier, J. (1998). Gamma-band electroencephalographic oscillations in a patient with somatic hallucinations. *Lancet, 352,* 620-621.

Barres, B. A., Jacobson, M. D., Schmid, R., Sendtner, M., & Raff, M. C. (1993). Does oligodendrocyte survival depend on axons? *Current Biology, 3,* 489-497.

Bartzokis, G. (2002). Schizophrenia: breakdown in the well-regulated lifelong process of brain development and maturation. *Neuropsychopharmacology, 27,* 672-683.

Bartzokis, G., Beckson, M., Lu, P. H., Nuechterlein, K. H., Edwards, N., & Mintz, J. (2001). Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study. *Archives of General Psychiatry, 58,* 461-465.

Bear, M. F., Connors, B. W., & Paradiso, M. A. (1996). *Neuroscience: Exploring the Brain*. Baltimore: Williams and Wilkins.

Beaumont, G. (2000). Antipsychotics--the future of schizophrenia treatment. *Current Medical Research & Opinion, 16,* 37-42.

Begic, D., Hotujac, L., & Jokic-Begic, N. (2000a). Quantitative EEG in 'positive' and 'negative' schizophrenia. *Acta Psychiatrica Scandinavica, 101,* 307-311.

Begic, D., Hotujac, L., & Jokic-Begic, N. (2000b). Quantitative EEG in schizophrenic patients before and during pharmacotherapy. *Neuropsychobiology, 41,* 166-170.

Benes, F. M. (1989). Myelination of cortical-hippocampal relays during late adolescence. *Schizophrenia Bulletin, 15,* 585-593.

Benes, F. M., Turtle, M., Khan, Y., & Farol, P. (1994). Myelination of a key relay zone in the hippocampal formation occurs in the human brain during childhood, adolescence, and adulthood. *Archives of General Psychiatry, 51,* 477-484.

Berlim, M. T., Mattevi, B. S., Belmonte-de-Abreu, P., & Crow, T. J. (2003). The etiology of schizophrenia and the origin of language: overview of a theory. *Comprehensive Psychiatry, 44,* 7-14.

Bersani, G., Iannitelli, A., Maselli, P., Pancheri, P., Aloe, L., Angelucci, F. et al. (1999). Low nerve growth factor plasma levels in schizophrenic patients: a preliminary study. *Schizophrenia Research, 37,* 201-203.

Bilder, R. M., Lipschutz-Broch, L., Reiter, G., Geisler, S. H., Mayerhoff, D. I., & Lieberman, J. A. (1992). Intellectual deficits in first-episode schizophrenia: evidence for progressive deterioration. *Schizophrenia Bulletin, 18,* 437-438.

Birley, J. L. & Brown, G. W. (1970). Crises and life changes preceding the onset or relapse of acute schizophrenia: clinical aspects. *British Journal of Psychiatry, 116,* 327-333.

Blakemore, S. J., Wolpert, D. M., & Frith, C. D. (1998). Central cancellation of self-produced tickle sensation. *Nature Neuroscience, 1,* 635-640.

Bland, M. J. & Altman, D. G. (1995). Statistics notes: Multiple significance tests: the Bonferroni method. *British Medical Journal, 310,* 170.

Bleuler, E. (1911). *Dementia Praecox or the Group of Schizophrenias*. New York: International Universities Press.

Bogerts, B. (1999). The neuropathology of schizophrenic diseases: historical aspects and present knowledge. *European Archives of Psychiatry & Clinical Neuroscience, 249,* 2-13.

Bogerts, B., Ashtari, M., Degreef, G., Alvir, J. M., Bilder, R. M., & Lieberman, J. A. (1990). Reduced temporal limbic structure volumes on magnetic resonance images in first episode schizophrenia. *Psychiatry Research, 35,* 1-13.

Bookstein, F. L. (2001). "Voxel-based morphometry" should not be used with imperfectly registered images. *NeuroImage, 14,* 1454-1462.

Bosanac, P., Norman, T., Burrows, G., & Beumont, P. (2005). Serotonergic and dopaminergic systems in anorexia nervosa: a role for atypical antipsychotics? *Australian & New Zealand Journal of Psychiatry, 39,* 146-153.

Bourgeois, J. P. & Rakic, P. (1993). Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. *Journal of Neuroscience, 13,* 2801-2820.

Braitenberg, V. (1991). *Anatomy of the cortex: statistics and geometry*. Berlin: Springer-Verlag.

Bressler, S. L. (1995). Large-scale cortical networks and cognition. *Brain Research Reviews, 20,* 288-304.

Brooks, D. J. (2000). Morphological and functional imaging studies on the diagnosis and progression of Parkinson's disease. *Journal of Neurology, 247,* 11-18.

Buchanan, R. W., Vladar, K., Barta, P. E., & Pearlson, G. D. (1998). Structural evaluation of the prefrontal cortex in schizophrenia. *American Journal of Psychiatry, 155,* 1049-1055.

Bullmore, E. T., Frangou, S., & Murray, R. M. (1997). The dysplastic net hypothesis: an integration of developmental and dysconnectivity theories of schizophrenia. *Schizophrenia Research, 28,* 143-156.

Bullmore, E. T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E., & Brammer, M. J. (1999). Global, voxel and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Transactions on Medical Imaging, 18,* 32-42.

Cabeza, R. & Nyberg, L. (2000). Imaging cognition II: An empirical review of 275 PET and fMRI studies. *Journal of Cognitive Neuroscience, 12,* 1-47.

Cahn, W., Hulshoff Pol, H. E., Bongers, M., Schnack, H. G., Mandl, R. C., van Haren, N. E. et al. (2002a). Brain morphology in antipsychotic-naive schizophrenia: a study of multiple brain structures. *British Journal of Psychiatry - Supplementum, 43,* s66-72.

Cahn, W., Pol, H. E., Lems, E. B., van Haren, N. E., Schnack, H. G., van der Linden, J. A. et al. (2002b). Brain volume changes in first-episode schizophrenia: a 1-year follow-up study. *Archives of General Psychiatry, 59,* 1002-1010.

Carbone, S., Harrigan, S., McGorry, P. D., Curry, C., & Elkins, K. (1999). Duration of untreated psychosis and 12-month outcome in first-episode psychosis: the impact of treatment approach. *Acta Psychiatrica Scandinavica, 100,* 96-104.

Carlson, N. R. (2002). *Foundations of physiological psychology*. Boston, London: Allyn and Bacon.

Carr, V. J., Lewin, T. J., Neil, A. L., Halpin, S. A., & Holmes, S. (2004). Premorbid, psychosocial and clinical predictors of the costs of schizophrenia and other psychoses. *British Journal of Psychiatry, 184,* 517-525.

Carr, V. J., Neil, A. L., Halpin, S. A., Holmes, S., & Lewin, T. J. (2003). Costs of schizophrenia and other psychoses in urban Australia: findings from the Low Prevalence (Psychotic) Disorders Study. *Australian & New Zealand Journal of Psychiatry, 37,* 31-40.

Chau, W. & McIntosh, A. R. (2005). The Talairach coordinate of a point in the MNI space: how to interpret it. *NeuroImage, 25,* 408-416.

Chen, S. H. & Desmond, J. E. (2005). Temporal dynamics of cerebro-cerebellar network recruitment during a cognitive task. *Neuropsychologia, 43,* 1227-1237.

Christensen, G. E., Rabbitt, R. D., & Miller, M. I. (1996). Deformable templates using large deformation kinematics. *IEEE Transactions on Image Processing, 5,* 1435-1447.

Chua, S. E., Wright, I. C., Poline, J.-B., Liddle, P. F., Murray, R. M., Frackowiak, R. S. J. et al. (1997). Grey matter correlates of syndromes in schizophrenia: a semi automated analysis of structural magnetic resonance images. *British Journal of Psychiatry, 170,* 406-410.

Close, H. & Garety, P. (1998). Cognitive assessment of voices: further developments in understanding the emotional impact of voices. *British Journal of Clinical Psychology, 37,* 173-188.

Corson, P. W., Nopoulos, P., Miller, D. D., Arndt, S., & Andreasen, N. C. (1999). Change in basal ganglia volume over 2 years in patients with schizophrenia: typical versus atypical neuroleptics. *American Journal of Psychiatry, 156,* 1200-1204.

Craig, T. K. & Timms, P. W. (1995). Homelessness and schizophrenia. In S.R.Hirsch & D. R. Weinberger (Eds.), *Schizophrenia* (pp. 664-684). Oxford: Blackwell Science.

Crow, T. J. (1995). A continuum of psychosis, one human gene, and not much else--the case for homogeneity. *Schizophrenia Research, 17,* 135-145.

Crow, T. J. (1997). Schizophrenia as failure of hemispheric dominance for language. *Trends in Neurosciences., 20,* 339-343.

Cuesta, M. J., Peralta, V., & Zarzuela, A. (1998). Illness duration and neuropsychological impairments in schizophrenia. *Schizophrenia Research, 33,* 141-150.

Cullberg, J. (2003). Stressful life events preceding the first onset of psychosis. An explorative study. *Nordic Journal of Psychiatry, 57,* 209-214.

Cutting, J. (1985). *The psychology of schizophrenia*. Edinburgh: Churchill Livingstone.

Cutting, J. (1995). Descriptive Psychopathology. In S.R.Hirsch & D. R. Weinberger (Eds.), *Schizophrenia* (pp. 15-27). Oxford: Blackwell Science.

Davidson, M. & Davis, K. L. (1988). A comparison of plasma homovanillic acid concentrations in schizophrenic patients and normal controls. *Archives of General Psychiatry, 45,* 561-563.

Davis, J. M. (1974). Dose equivalence of the antipsychotic drugs. *Journal of Psychiatric Research, 11,* 69.

Davis, K. L., Kahn, R. S., Ko, G., & Davidson, M. (1991). Dopamine in schizophrenia: a review and reconceptualization. *American Journal of Psychiatry, 148,* 1474-1486.

Davis, K. L., Stewart, D. G., Friedman, J. I., Buchsbaum, M., Harvey, P. D., Hof, P. R. et al. (2003). White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Archives of General Psychiatry, 60,* 443-456.

Davison, G. C. & Neale, J. M. (1998). *Abnormal Psychology*. New York: John Wiley and Sons, Inc.

Day, R. (1981). Life events and schizophrenia: the "triggering" hypothesis. *Acta Psychiatrica Scandinavica, 64,* 97-122.

DeLisi, L. E., Dauphinais, I. D., & Gershon, E. S. (1988). Perinatal complications and reduced size of brain limbic structures in familial schizophrenia. *Schizophrenia Bulletin, 14,* 185-191.

DeLisi, L. E. & Hoff, A. L. (2005). Failure to find progressive temporal lobe volume decreases 10 years subsequent to a first episode of schizophrenia. *Psychiatry Research, 138,* 265-268.

DeLisi, L. E., Sakuma, M., Maurizio, A. M., Relja, M., & Hoff, A. L. (2004). Cerebral ventricular change over the first 10 years after the onset of schizophrenia. *Psychiatry Research, 130,* 57-70.

DeLisi, L. E., Stritzke, P., Riordan, H., Holan, V., Boccio, A., Kushner, M. et al. (1992). The timing of brain morphological changes in schizophrenia and their relationship to clinical outcome. *Biological Psychiatry, 31,* 241-254.

Diwadkar, V. A., DeBellis, M. D., Sweeney, J. A., Pettegrew, J. W., & Keshavan, M. S. (2004). Abnormalities in MRI-measured signal intensity in the corpus callosum in schizophrenia. *Schizophrenia Research, 67,* 277-282.

Dolan, R. J., Fletcher, P., Frith, C. D., Friston, K. J., Frackowiak, R. S., & Grasby, P. M. (1995). Dopaminergic modulation of impaired cognitive activation in the anterior cingulate cortex in schizophrenia. *Nature, 378,* 180-182.

Drayer, B. P. (1988). Imaging of the aging brain. Part I. Normal findings. *Radiology, 166,* 785-796.

Duckworth, K., Halpern, J. H., Schutt, R. K., & Gillespie, C. (2003). Use of schizophrenia as a metaphor in US newspapers. *Psychiatric Services, 54,* 1402-1404.

Dunlap, C. B. (1924). Dementia Praecox. Some Preliminary Observations on Brains from Carefully Selected Cases and a Consideration of Certain Sources of Error. *American Journal of Psychiatry, 3,* 403-421.

Dustman, R. E., Shearer, D. E., & Emmerson, R. Y. (1999). Life-span changes in EEG spectral amplitude, amplitude variability and mean frequency. *Clinical Neurophysiology, 110,* 1399-1409.

Editorial (1988). Where next with psychiatric illness? *Nature, 336,* 95-96.

Egan, M. F., Goldberg, T. E., Kolachana, B. S., Callicott, J. H., Mazzanti, C. M., Straub, R. E. et al. (2001). Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America, 98,* 6917-6922.

Engel, A. K., Roelfsema, P. R., Fries, P., Brecht, M., & Singer, W. (1997). Role of the temporal domain for response selection and perceptual binding. *Cerebral Cortex, 7,* 571-582.

Evans, A. C., Kambler, M., Collins, D. L., & Macdonald, D. (1994). An MRI-based probabilistic atlas of neuroanatomy. In S.Shorvon, D. Fish, F. Andermann, G. M. Bydder, & H. Stefan (Eds.), *Magnetic Resonance Scanning and Epilepsy* (New York: NATO ASI Series A, Life Sciences, Vol. 264. Plenum.

Falkai, P. & Bogerts, B. (1995). The Neuropathology of Schizophrenia. In S.R.Hirsch & D. R. Weinberger (Eds.), *Schizophrenia* (pp. 275-292). Oxford: Blackwell Science.

Fannon, D., Chitnis, X., Doku, V., Tennakoon, L., O'Ceallaigh, S., Soni, W. et al. (2000). Features of structural brain abnormality detected in first-episode psychosis. *American Journal of Psychiatry, 157,* 1829-1834.

Farrow, T. F., Whitford, T. J., Williams, L. M., Gomes, L., & Harris, A. W. F. (2005). Diagnosis-related regional grey matter loss over two years in first episode schizophrenia and bipolar disorder. *Biological Psychiatry, 58,* 713-723.

Feinberg, I. (1982). Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *Journal of Psychiatric Research, 17,* 319-334.

Fenton, W. S. & McGlashan, T. H. (1991). Natural history of schizophrenia subtypes. I. Longitudinal study of paranoid, hebephrenic, and undifferentiated schizophrenia. *Archives of General Psychiatry, 48,* 969-977.

Filipek, P. A., Richelme, C., Kennedy, D. N., & Caviness, V. S., Jr. (1994). The young adult human brain: an MRI-based morphometric analysis. *Cerebral Cortex, 4,* 344-360.

Filippi, M., Cercignani, M., Inglese, M., Horsfield, M. A., & Comi, G. (2001). Diffusion tensor magnetic resonance imaging in multiple sclerosis. *Neurology, 56,* 304-311.

Fitzgerald, D., Lucas, S., Redoblado, M. A., Winter, V., Brennan, J., Anderson, J. et al. (2004). Cognitive functioning in young people with first episode psychosis: relationship to diagnosis and clinical characteristics. *Australian & New Zealand Journal of Psychiatry, 38,* 501-510.

Flynn, S. W., Lang, D. J., Mackay, A. L., Goghari, V., Vavasour, I. M., Whittall, K. P. et al. (2003). Abnormalities of myelination in schizophrenia detected in vivo with MRI, and post-mortem with analysis of oligodendrocyte proteins. *Molecular Psychiatry, 8,* 811-820.

Foong, J., Maier, M., Barker, G. J., Brocklehurst, S., Miller, D. H., & Ron, M. A. (2000). In vivo investigation of white matter pathology in schizophrenia with magnetisation transfer imaging. *Journal of Neurology, Neurosurgery & Psychiatry, 68,* 70-74.

Forger, N. G. & Breedlove, S. M. (1986). Sexual dimorphism in human and canine spinal cord: role of early androgen. *Proceedings of the National Academy of Sciences of the United States of America, 83,* 7527-7531.

Freeborough, P. A. & Fox, N. C. (1998). Modeling brain deformations in Alzheimer disease by fluid registration of serial 3D MR images. *Journal of Computer Assisted Tomography, 22,* 838-843.

Friedman, J. H. & Factor, S. A. (2000). Atypical antipsychotics in the treatment of drug-induced psychosis in Parkinson's disease. *Movement Disorders, 15,* 201-211.

Friedmann, B. R., Jones, J. P., Chavez-Munoz, G., Salmon, A. P., & Merritt, C. R. (1989). *Principles of MRI.* New York: McGraw-Hill.

Friston, K. (1998). The disconnection hypothesis. *Schizophrenia Research, 30,* 115-125.

Friston, K. (2004). Introduction: experimental design and statistical parametric mapping. In R.S.Frackowiak, J. Ashburner, K. Friston, & W. Penny (Eds.), *Human brain function* (pp. 1-67). Amsterdam, Boston: Elselvier Academic Press.

Friston, K. J., Ashburner, J., Frith, C. D., Poline, J.-B., Heather, J. D., & Frackowiak, R. S. J. (1995). Spatial registration and normalization of images. *Human Brain Mapping, 2,* 189-210.

Friston, K. J., Frith, C. D., Liddle, P. F., & Frackowiak, R. S. (1991). Comparing functional (PET) images: the assessment of significant change. *Journal of Cerebral Blood Flow & Metabolism, 11,* 690-699.

Frith, C. (2005). The neural basis of hallucinations and delusions. *Comptes Rendus Biologies, 328,* 169-175.

Frith, C. D. (1992). *The cognitive neuropsychology of schizophrenia*. Hove, UK: Lawrence Erlbaum Associates.

Frith, C. D., Blakemore, S., & Wolpert, D. M. (2000). Explaining the symptoms of schizophrenia: abnormalities in the awareness of action. *Brain Research Reviews, 31,* 357-363.

Frith, C. D. & Done, D. J. (1989). Experiences of alien control in schizophrenia reflect a disorder in the central monitoring of action. *Psychological Medicine, 19,* 359-363.

Fromm-Reichmann, F. (1948). Notes on the development of treatment of schizophrenics by psychoanalytic psychotherapy. *Psychiatry, 11,* 263-273.

Frumin, M., Golland, P., Kikinis, R., Hirayasu, Y., Salisbury, D. F., Hennen, J. et al. (2002). Shape differences in the corpus callosum in first-episode schizophrenia and first-episode psychotic affective disorder. *American Journal of Psychiatry, 159,* 866-868.

Garmezy, N. & Streitman, S. (1974). Children at risk: The search for the antecedents of schizophrenia: I. Conceptual models and research methods. *Schizophrenia Bulletin, 8,* 14-90.

Gasser, T., Verleger, R., Bacher, P., & Sroka, L. (1988). Development of the EEG of school-age children and adolescents. I. Analysis of band power. *Electroencephalography & Clinical Neurophysiology, 69*, 91-99.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A. et al. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience, 2*, 861-863.

Giuliani, N. R., Calhoun, V. D., Pearlson, G. D., Francis, A., & Buchanan, R. W. (2005). Voxel-based morphometry versus region of interest: a comparison of two methods for analyzing gray matter differences in schizophrenia. *Schizophrenia Research, 74*, 135-147.

Good, C. D., Johnsrude, I., Ashburner, J., Henson, R. N. A., Friston, K. J., & Frackowiak, R. S. J. (2001). A voxel-based morphometric study of aging in 465 normal adult human brains. *NeuroImage, 14*, 21-36.

Gordon, E. (2003). Integrative neuroscience in psychiatry: the role of a standardized database. *Australasian Psychiatry, 11*, 156-163.

Gordon, E., Cooper, N., Rennie, C., Hermens, D., & Williams, L. M. (2005). Integrative neuroscience: the role of a standardized database. *Clinical EEG & Neuroscience: Official Journal of the EEG & Clinical Neuroscience Society (ENCS), 36*, 64-75.

Gordon, E., Williams, L. M., Haig, A. R., Bahramali, H., Wright, J. J., & Mears, R. (2001). Symptom profile and "gamma" processing in schizophrenia. *Cognitive Neuropsychiatry, 6*, 7-20.

Gottesman, I. I., McGuffin, P., & Farmer, A. E. (1987). Clinical genetics as clues to the "real" genetics of schizophrenia (a decade of modest gains while playing for time). *Schizophrenia Bulletin, 13*, 23-47.

Gottwald, B., Wilde, B., Mihajlovic, Z., & Mehdorn, H. M. (2004). Evidence for distinct cognitive deficits after focal cerebellar lesions. *Journal of Neurology, Neurosurgery & Psychiatry, 75*, 1524-1531.

Grant, D. A. & Berg, E. A. (1948). A behavioral analysis of degree of reinforcement and ease of shifting to new responses in a Weigl-type card sorting problem. *Journal of Experimental Psychology, 34*, 404-411.

Gratton, G., Coles, M., & Donchin, E. (1983). A new method for the off-line removal of ocular artifact. *Electroencephalography and Clinical Neurophysiology, 55,* 468-484.

Gray, J. A. (1998). Integrating schizophrenia. *Schizophrenia Bulletin, 24,* 249-268.

Green, A. I., Salomon, M. S., Brenner, M. J., & Rawlins, K. (2002). Treatment of schizophrenia and comorbid substance use disorder. *Current Drug Targets - CNS & Neurological Disorders, 1,* 129-139.

Grieve, S. M., Clark, R. C., Williams, L. M., Peduto, A., & Gordon, E. (2005). Preservation of limbic and paralimbic structures in aging. *Human Brain Mapping, 25,* 391-401.

Guillem, F., Bicu, M., Pampoulova, T., Hooper, R., Bloom, D., Wolf, M. A. et al. (2003). The cognitive and anatomo-functional basis of reality distortion in schizophrenia: a view from memory event-related potentials. *Psychiatry Research, 117,* 137-158.

Gunduz, H., Wu, H., Ashtari, M., Bogerts, B., Crandall, D., Robinson, D. G. et al. (2002). Basal ganglia volumes in first-episode schizophrenia and healthy comparison subjects. *Biological Psychiatry, 51,* 801-808.

Gunther, W., Petsch, R., Steinberg, R., Moser, E., Streck, P., Heller, H. et al. (1991). Brain dysfunction during motor activation and corpus callosum alterations in schizophrenia measured by cerebral blood flow and magnetic resonance imaging. *Biological Psychiatry, 29,* 535-555.

Gur, R. E., Cowell, P., Turetsky, B. I., Gallacher, F., Cannon, T., Bilker, W. B. et al. (1998a). A follow-up magnetic resonance imaging study of schizophrenia: relationship of neuroanatomical changes to clinical and neurobehavioral measures. *Archives of General Psychiatry, 55,* 145-152.

Gur, R. E., Cowell, P. E., Latshaw, A., Turetsky, B. I., Grossman, R. I., Arnold, S. E. et al. (2000). Reduced dorsal and orbital prefrontal gray matter volumes in schizophrenia. *Archives of General Psychiatry, 57,* 761-768.

Gur, R. E., Maany, V., Mozley, D., Swanson, C., Bilker, W., & Gur, R. C. (1998b). Subcortical MRI volumes in neuroleptic-naive and treated patients with schizophrenia. *American Journal of Psychiatry, 155,* 1711-1717.

Gur, R. E., Maany, V., Mozley, P. D., Swanson, C., Bilker, W., & Gur, R. C. (1998c). Subcortical MRI volumes in neuroleptic-naive and treated patients with schizophrenia. *American Journal of Psychiatry, 155,* 1711-1717.

Guy, W. (1976). Clinical Global Impressions. In National Institute of Mental Health (Ed.), *ECDEU Assessment Manual for Psychopharmacology, Revised* (pp. 218-222). Rockville.

Hakak, Y., Walker, J. R., Li, C., Wong, W. H., Davis, K. L., Buxbaum, J. D. et al. (2001). Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America, 98,* 4746-4751.

Hambrecht, M. & Hafner, H. (1996). Substance abuse and the onset of schizophrenia. *Biological Psychiatry, 40,* 1155-1163.

Hammers, A., Allom, R., Koepp, M. J., Free, S. L., Myers, R., Lemieux, L. et al. (2003). Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. *Human Brain Mapping.,* 224-247.

Harasty, J., Double, K. L., Halliday, G. M., Kril, J. J., & McRitchie, D. A. (1997). Language-associated cortical regions are proportionally larger in the female brain. *Archives of Neurology, 54,* 171-176.

Harris, A., Brennan, J., Anderson, J., Taylor, A., Sanbrook, M., Fitzgerald, D. et al. (2005). Clinical profiles, scope and general findings of the Western Sydney First Episode Psychosis Project. *Australian & New Zealand Journal of Psychiatry, 39,* 36-43.

Harris, A., Gordon, E., Anderson, J., Ritchie, G., McLachlan, C., & Meares, R. (1997). Change in quantified electroencephalography (QEEG) with medication and altered clinical state in the same subjects with schizophrenia. *Schizophrenia Research, 23,* 87-89.

Harris, A. W. F., Williams, L. M., Gordon, E., Bahramali, H., & Slewa-Younan, S. (1999). Different psychopathological models and quantified EEG in schizophrenia. *Psychological Medicine, 29,* 1175-1181.

Harris, E. C. & Barraclough, B. (1997). Suicide as an outcome for mental disorders. A meta-analysis. *British Journal of Psychiatry, 170,* 205-228.

Hartwell, C. E. (1996). The schizophrenogenic mother concept in American psychiatry. *Psychiatry: Interpersonal and Biological Processes, 59,* 274-297.

Hedges, D., Jeppson, K., & Whitehead, P. (2003). Antipsychotic medication and seizures: a review. *Drugs of Today, 39,* 551-557.

Hegarty, J. D., Baldessarini, R. J., Tohen, M., Waternaux, C., & Oepen, G. (1994). One hundred years of schizophrenia: a meta-analysis of the outcome literature. *American Journal of Psychiatry, 151,* 1409-1416.

Henderson, C. E. (1996). Programmed cell death in the developing nervous system. *Neuron, 17,* 579-585.

Hendry, I. A. (1975). The response of adrenergic neurones to axotomy and nerve growth factor. *Brain Research, 94,* 87-97.

Herman, D. B., Susser, E. S., Jandorf, L., Lavelle, J., & Bromet, E. J. (1998). Homelessness among individuals with psychotic disorders hospitalized for the first time: findings from the Suffolk County Mental Health Project. *American Journal of Psychiatry, 155,* 109-113.

Herz, M. (1997). Practice guidelines for the treatment of patients with schizophrenia. *American Journal of Psychiatry, 154,* 10.

Hickie, I., Hadzi-Pavlovic, D., Scott, E., Davenport, T., Koschera, A., & Naismith, S. (1998). SPHERE: A National Depression Project. *Australasian Psychiatry, 6,* 248-250.

Hirayasu, Y., Shenton, M. E., Salisbury, D. F., Dickey, C. C., Fischer, I. A., Mazzoni, P. et al. (1998). Lower left temporal lobe MRI volumes in patients with first-episode schizophrenia compared with psychotic patients with first-episode affective disorder and normal subjects. *American Journal of Psychiatry, 155,* 1384-1391.

Hirayasu, Y., Tanaka, S., Shenton, M. E., Salisbury, D. F., DeSantis, M. A., Levitt, J. J. et al. (2001). Prefrontal gray matter volume reduction in first episode schizophrenia. *Cerebral Cortex, 11,* 374-381.

Ho, B. C., Andreasen, N. C., Nopoulos, P., Arndt, S., Magnotta, V., & Flaum, M. (2003). Progressive structural brain abnormalities and their relationship to clinical

outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. *Archives of General Psychiatry, 60,* 585-594.

Hof, P. R., Haroutunian, V., Friedrich, V. L., Jr., Byne, W., Buitron, C., Perl, D. P. et al. (2003). Loss and altered spatial distribution of oligodendrocytes in the superior frontal gyrus in schizophrenia. *Biological Psychiatry, 53,* 1075-1085.

Hoff, A. L., Sakuma, M., Wieneke, M., Horon, R., Kushner, M., & DeLisi, L. E. (1999). Longitudinal neuropsychological follow-up study of patients with first-episode schizophrenia. *American Journal of Psychiatry, 156,* 1336-1341.

Hoffman, R. E. & Dobscha, S. K. (1989). Cortical pruning and the development of schizophrenia: a computer model. *Schizophrenia Bulletin, 15,* 477-490.

Hoffman, R. E. & McGlashan, T. H. (2001). Neural network models of schizophrenia. *Neuroscientist, 7,* 441-454.

Honea, R., Crow, T. J., Passingham, D., & Mackay, C. E. (2005). Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. *American Journal of Psychiatry, 162,* 2233-2245.

Hounsfield, G. N. (1973). Computerized transverse axial scanning (tomography). 1. Description of system. *British Journal of Radiology, 46,* 1016-1022.

Hulshoff Pol, H. E., Hilleke, E., Schnack, H. G., Bertens, M., van Haren, N., van der Tweel, I. et al. (2002). Volume changes in gray matter in patients with schizophrenia. *American Journal of Psychiatry, 159,* 244-250.

Hulshoff Pol, H. E., Schnack, H. G., Mandl, R. C., Cahn, W., Collins, D. L., Evans, A. C. et al. (2004). Focal white matter density changes in schizophrenia: reduced inter-hemispheric connectivity. *NeuroImage, 21,* 27-35.

Hulshoff Pol, H. E., Schnack, H. G., Mandl, R. C., van Haren, N. E., Koning, H., Collins, D. L. et al. (2001). Focal gray matter density changes in schizophrenia. *Archives of General Psychiatry, 58,* 1118-1125.

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Research, 163,* 195-205.

Huttenlocher, P. R. (1999). Dendritic and synaptic development in human cerebral cortex: time course and critical periods. *Developmental Neuropsychology, 16,* 347-349.

Huttenlocher, P. R. & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology, 387,* 167-178.

Ichimiya, T., Okubo, Y., Suhara, T., & Suda, Y. (2001). Reduced volume of the cerebellar vermis in neuroleptic-naive schizophrenia. *Biological Psychiatry, 49,* 20-27.

Jablensky, A. (1997). The 100-year epidemiology of schizophrenia. *Schizophrenia Research, 28,* 111-125.

Jayakumar, P. N., Venkatasubramanian, G., Gangadhar, B. N., Janakiramaiah, N., & Keshavan, M. S. (2005). Optimized voxel-based morphometry of gray matter volume in first-episode, antipsychotic-naive schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29,* 587-591.

Job, D. E., Whalley, H. C., McConnell, S., Glabus, M., Johnstone, E. C., & Lawrie, S. M. (2002). Structural gray matter differences between first-episode schizophrenics and normal controls using voxel-based morphometry. *NeuroImage, 17,* 880-889.

Johnstone, E. C., Crow, T. J., Frith, C. D., Husband, J., & Kreel, L. (1976). Cerebral ventricular size and cognitive impairment in chronic schizophrenia. *Lancet, 2,* 924-926.

Jones, P., Rodgers, B., Murray, R., & Marmot, M. (1994). Child development risk factors for adult schizophrenia in the British 1946 birth cohort. *Lancet, 344,* 1398-1402.

Joyal, C. C., Laakso, M. P., Tiihonen, J., Syvalahti, E., Vilkman, H., Laakso, A. et al. (2002). A volumetric MRI study of the entorhinal cortex in first episode neuroleptic-naive schizophrenia. *Biological Psychiatry, 51,* 1005-1007.

Joyal, C. C., Laakso, M. P., Tiihonen, J., Syvalahti, E., Vilkman, H., Laakso, A. et al. (2003). The amygdala and schizophrenia: a volumetric magnetic resonance imaging study in first-episode, neuroleptic-naive patients. *Biological Psychiatry, 54,* 1302-1304.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). *Principles of neural science*. (4 ed.) New York: McGraw Hill.

Kane, J. M. (1989). The current status of neuroleptic therapy. *Journal of Clinical Psychiatry, 50,* 322-328.

Kanner, A. M. (2004). Structural MRI changes of the brain in depression. *Clinical EEG & Neuroscience: Official Journal of the EEG & Clinical Neuroscience Society (ENCS), 35,* 46-52.

Kaplan, R. D., Szechtman, H., Franco, S., Szechtman, B., Nahmias, C., Garnett, E. S. et al. (1993). Three clinical syndromes of schizophrenia in untreated subjects: relation to brain glucose activity measured by positron emission tomography (PET). *Schizophrenia Research, 11,* 47-54.

Kapur, S. & Mamo, D. (2003). Half a century of antipsychotics and still a central role for dopamine D2 receptors. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27,* 1081-1090.

Kasai, K., Iwanami, A., Yamasue, H., Kuroki, N., Nakagome, K., & Fukuda, M. (2002). Neuroanatomy and neurophysiology of schizophrenia. *Neuroscience Research, 43,* 93-110.

Kasai, K., Shenton, M. E., Salisbury, D. F., Hirayasu, Y., Onitsuka, T., Spencer, M. H. et al. (2003). Progressive decrease of left Heschl gyrus and planum temporale gray matter volume in first-episode schizophrenia: a longitudinal magnetic resonance imaging study. *Archives of General Psychiatry, 60,* 766-775.

Kassubek, J., Bernhard, L. G., Ecker, D., Juengling, F. D., Muche, R., Schuller, S. et al. (2004). Global cerebral atrophy in early stages of Huntington's disease: quantitative MRI study. *NeuroReport, 15,* 363-365.

Kay, S. R., Opler, L. A., & Lindenmayer, J.-P. (1989). The Positive and Negative Syndrome Scale (PANSS): Rationale and Standardisation. *British Journal of Psychiatry, 155,* 59-65.

Kendler, K. S., Heninger, G. R., & Roth, R. H. (1981). Brain contribution to the haloperidol-induced increase in plasma homovanillic acid. *European Journal of Pharmacology, 71,* 321-326.

Kendler, K. S., Heninger, G. R., & Roth, R. H. (1982). Influence of dopamine agonists on plasma and brain levels of homovanillic acid. *Life Sciences, 30,* 2063-2069.

Kendler, K. S., McGuire, M., Gruenberg, A. M., & Walsh, D. (1994). Outcome and family study of the subtypes of schizophrenia in the west of Ireland. *American Journal of Psychiatry, 151,* 849-856.

Keshavan, M. S. (1999). Development, disease and degeneration in schizophrenia: a unitary pathophysiological model. *Journal of Psychiatric Research, 33,* 513-521.

Keshavan, M. S., Anderson, S., & Pettegrew, J. W. (1994). Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex? The Feinberg hypothesis revisited. *Journal of Psychiatric Research, 28,* 239-265.

Keshavan, M. S., Haas, G. L., Kahn, C. E., Aguilar, E., Dick, E. L., Schooler, N. R. et al. (1998). Superior temporal gyrus and the course of early schizophrenia: progressive, static, or reversible? *Journal of Psychiatric Research, 32,* 161-167.

Kim, J., Crespo-Facorro, B., Andreasen, N. C., O'Leary, D. S., Magnotta, V., & Nopoulos, P. C. (2003). Morphology of the lateral superior temporal gyrus in neuroleptic naive patients with schizophrenia: relationship to symptoms. *Schizophrenia Research, 60,* 173-181.

Kim, S. G., Ugurbil, K., & Strick, P. L. (1994). Activation of a cerebellar output nucleus during cognitive processing. *Science, 265,* 949-951.

Kipps, C. M., Duggins, A. J., Mahant, N., Gomes, L., Ashburner, J., & McCusker, E. A. (2005). Progression of structural neuropathology in preclinical Huntington's disease: a tensor based morphometry study. *Journal of Neurology, Neurosurgery, and Psychiatry, 76,* 650-655.

Klyushnik, T. P., Danilovskaya, E. V., Vatolkina, O. E., Turkova, I. L., Tsutsul'kovskaya, M. Y., Orlova, V. A. et al. (1999). Changes in the serum levels of autoantibody to nerve growth factor in patients with schizophrenia. *Neuroscience & Behavioral Physiology, 29,* 355-357.

Kraemer, H. C., Yesavage, J. A., Taylor, J. L., & Kupfer, D. (2000). How can we learn about developmental processes from cross-sectional studies, or can we? *American Journal of Psychiatry, 157,* 163-171.

Kubicki, M., Park, H., Westin, C. F., Nestor, P. G., Mulkern, R. V., Maier, S. E. et al. (2005). DTI and MTR abnormalities in schizophrenia: analysis of white matter integrity. *NeuroImage, 26,* 1109-1118.

Kubicki, M., Shenton, M. E., Salisbury, D. F., Hirayasu, Y., Kasai, K., Kikinis, R. et al. (2002). Voxel-based morphometric analysis of grey matter in first episode schizophrenia. *NeuroImage, 17,* 1711-1719.

Kuida, K., Zheng, T. S., Na, S., Kuan, C., Yang, D., Karasuyama, H. et al. (1996). Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature, 384,* 368-372.

Kuipers, E., Holloway, F., Rabe-Hesketh, S., Tennakoon, L., & Croydon Outreach and Assertive Support Team (COAST) (2004). An RCT of early intervention in psychosis: Croydon Outreach and Assertive Support Team (COAST). *Social Psychiatry & Psychiatric Epidemiology, 39,* 358-363.

Laakso, M. P., Tiihonen, J., Syvalahti, E., Vilkman, H., Laakso, A., Alakare, B. et al. (2001). A morphometric MRI study of the hippocampus in first-episode, neuroleptic-naive schizophrenia. *Schizophrenia Research, 50,* 3-7.

Lang, D. J., Kopala, L. C., Vandorpe, R. A., Rui, Q., Smith, G. N., Goghari, V. M. et al. (2001). An MRI study of basal ganglia volumes in first-episode schizophrenia patients treated with risperidone. *American Journal of Psychiatry, 158,* 625-631.

Lang, F. H., Forbes, J. F., Murray, G. D., & Johnstone, E. C. (1997). Service provision for people with schizophrenia. I. Clinical and economic perspective. *British Journal of Psychiatry, 171,* 159-164.

Lauriello, J., Hoff, A., Wieneke, M. H., Blankfeld, H., Faustman, W. O., Rosenbloom, M. et al. (1997). Similar extent of brain dysmorphology in severely ill women and men with schizophrenia. *American Journal of Psychiatry, 154,* 819-825.

Lee, B. C., Mintun, M., Buckner, R. L., & Morris, J. C. (2003). Imaging of Alzheimer's disease. *Journal of Neuroimaging, 13,* 199-214.

Lee, C. U., Shenton, M. E., Salisbury, D. F., Kasai, K., Onitsuka, T., Dickey, C. C. et al. (2002). Fusiform gyrus volume reduction in first-episode schizophrenia: a magnetic resonance imaging study. *Archives of General Psychiatry, 59,* 775-781.

Lee, K. H., Williams, L. M., Breakspear, M., & Gordon, E. (2003). Synchronous gamma activity: a review and contribution to an integrative neuroscience model of schizophrenia. *Brain Research Reviews, 41,* 57-78.

Lee, K. H., Williams, L. M., Haig, A. R., & Gordon, E. (2003). Gamma (40 Hz) phase synchronicity and symptom dimensions in schizophrenia. *Cognitive Neuropsychiatry, 8,* 57-71.

Lezak, M. D. (1995). *Neuropsychological assessment*. New York: Oxford University Press.

Liddle, P. F. (1987a). Schizophrenic syndromes, cognitive performance and neurological dysfunction. *Psychological Medicine, 17,* 49-57.

Liddle, P. F. (1987b). The symptoms of chronic schziophrenia: a re-examination of the positive-negative dichotamy. *British Journal of Psychiatry, 151,* 145-151.

Liddle, P. F., Friston, K. J., Frith, C. D., Hirsch, S. R., Jones, T., & Frackowiak, R. S. J. (1992). Patterns of cerebral blood flow in schizophrenia. *British Journal of Psychiatry, 160,* 179-186.

Lieberman, J., Chakos, M., Wu, H., Alvir, J., Hoffman, E., Robinson, D. et al. (2001). Longitudinal study of brain morphology in first episode schizophrenia. *Biological Psychiatry, 49,* 487-499.

Lieberman, J. A. (1999). Is schizophrenia a neurodegenerative disorder? A clinical and neurobiological perspective. *Biological Psychiatry, 46,* 729-739.

Lieberman, J. A., Kane, J. M., Gadaleta, D., Brenner, R., Lesser, M. S., & Kinon, B. (1984). Methylphenidate challenge as a predictor of relapse in schizophrenia. *American Journal of Psychiatry, 141,* 633-638.

Lieberman, J. A., Tollefson, G. D., Charles, C., Zipursky, R., Sharma, T., Kahn, R. S. et al. (2005). Antipsychotic drug effects on brain morphology in first-episode psychosis. *Archives of General Psychiatry, 62,* 361-370.

Lim, K. O., Adalsteinsson, E., Spielman, D., Sullivan, E. V., Rosenbloom, M. J., & Pfefferbaum, A. (1998). Proton magnetic resonance spectroscopic imaging of cortical gray and white matter in schizophrenia. *Archives of General Psychiatry, 55,* 346-352.

Linscott, R. J. (2005). Thought disorder, pragmatic language impairment, and generalized cognitive decline in schizophrenia. *Schizophrenia Research, 75,* 225-232.

Liu, X., Erikson, C., & Brun, A. (1996). Cortical synaptic changes and gliosis in normal aging, Alzheimer's disease and frontal lobe degeneration. *Dementia, 7,* 128-134.

Lovibond, P. F. & Lovibond, S. H. (1995). The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behaviour Research & Therapy, 33,* 335-343.

Lukoff, D., Snyder, K., Ventura, J., & Nuechterlein, K. H. (1984). Life events, familial stress, and coping in the developmental course of schizophrenia. *Schizophrenia Bulletin, 10,* 258-292.

Madsen, A. L., Karle, A., Rubin, P., Cortsen, M., Andersen, H. S., & Hemmingsen, R. (1999). Progressive atrophy of the frontal lobes in first-episode schizophrenia: interaction with clinical course and neuroleptic treatment. *Acta Psychiatrica Scandinavica, 100,* 367-374.

Marcelis, M., Suckling, J., Woodruff, P., Hofman, P., Bullmore, E., & van Os, J. (2003). Searching for a structural endophenotype in psychosis using computational morphometry. *Psychiatry Research, 122,* 153-167.

Margolis, R. L., Chuang, D. M., & Post, R. M. (1994). Programmed cell death: implications for neuropsychiatric disorders. *Biological Psychiatry, 35,* 946-956.

Martinovic, Z., Jovanovic, V., & Ristanovic, D. (1998). EEG power spectra of normal preadolescent twins. Gender differences of quantitative EEG maturation. *Neurophysiologie Clinique, 28,* 231-248.

Mathalon, D. H., Sullivan, E. V., Lim, K. O., & Pfefferbaum, A. (2001). Progressive brain volume changes and the clinical course of schizophrenia in men: a longitudinal magnetic resonance imaging study. *Archives of General Psychiatry, 58,* 148-157.

Matousek, M. & Petersen, I. (1973). Frequency analysis of the EEG in normal children and adolescents. In P.Kellaway & I. Petersen (Eds.), *Automation of clinical electroencephalography* (pp. 75-102). New York: Raven Press.

Matsuura, M., Yamamoto, K., Fukuzawa, H., Okubo, Y., Uesugi, H., Moriiwa, M. et al. (1985). Age development and sex differences of various EEG elements in healthy children and adults-quantification by a computerized wave form recognition method. *Electroencephalography & Clinical Neurophysiology, 60,* 394-406.

McCarley, R. W., Wible, C. G., Frumin, M., Hirayasu, Y., Levitt, J. J., Fisher, I. A. et al. (1999). MRI anatomy of schizophrenia. *Biological Psychiatry, 45,* 1099-1119.

McCreadie, R. G. (2003). Diet, smoking and cardiovascular risk in people with schizophrenia: descriptive study. *British Journal of Psychiatry, 183,* 534-539.

McGlashan, T. H. (1998). The profiles of clinical deterioration in schizophrenia. *Journal of Psychiatric Research, 32,* 133-141.

McGlashan, T. H. & Hoffman, R. E. (2000). Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Archives of General Psychiatry, 57,* 637-648.

McGrath, J. J., Hearle, J., Jenner, L., Plant, K., Drummond, A., & Barkla, J. M. (1999). The fertility and fecundity of patients with psychoses. *Acta Psychiatrica Scandinavica, 99,* 441-446.

McGuire, P. K. & Frith, C. D. (1996). Disordered functional connectivity in schizophrenia. *Psychological Medicine, 26,* 663-667.

McGuire, P. K., Silbersweig, D. A., Wright, I., Murray, R. M., Frackowiak, R. S. J., & Frith, C. D. (1996). The neural correlates of inner speech and auditory verbal imagery in schizophrenia: relationship to auditory verbal hallucinations. *British Journal of Psychiatry, 169,* 148-163.

Mellor, C. S. (1970). First rank symptoms of schizophrenia. I. The frequency in schizophrenics on admission to hospital. II. Differences between individual first rank symptoms. *British Journal of Psychiatry, 117,* 15-23.

Menon, R. R., Barta, P. E., Aylward, E. H., Richards, S. S., Vaughn, D. D., Tien, A. Y. et al. (1995). Posterior superior temporal gyrus in schizophrenia: grey matter changes and clinical correlates. *Schizophrenia Research, 16,* 127-135.

Middleton, F. A. & Strick, P. L. (1994). Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science, 266,* 458-461.

Milev, P., Ho, B. C., Arndt, S., & Andreasen, N. C. (2005). Predictive values of neurocognition and negative symptoms on functional outcome in schizophrenia: a longitudinal first-episode study with 7-year follow-up. *American Journal of Psychiatry, 162,* 495-506.

Milner, P. M. (1974). A model for visual shape recognition. *Psychological Review, 81,* 521-535.

Mion, C. C., Andreasen, N. C., Arndt, S., Swayze, V. W., & Cohen, G. A. (1991). MRI abnormalities in tardive dyskinesia. *Psychiatry Research, 40,* 157-166.

Molina, V., Sanz, J., Sarramea, F., Benito, C., & Palomo, T. (2004). Lower prefrontal gray matter volume in schizophrenia in chronic but not in first episode schizophrenia patients. *Psychiatry Research, 131,* 45-56.

Monkul, E. S., Malhi, G. S., & Soares, J. C. (2005). Anatomical MRI abnormalities in bipolar disorder: do they exist and do they progress?. [Review] [67 refs]. *Australian & New Zealand Journal of Psychiatry.39(4):222-6.*

Moorhead, T. W., Job, D. E., Whalley, H. C., Sanderson, T. L., Johnstone, E. C., & Lawrie, S. M. (2004). Voxel-based morphometry of comorbid schizophrenia and learning disability: analyses in normalized and native spaces using parametric and nonparametric statistical methods. *NeuroImage, 22,* 188-202.

Morihisa, J. M., Duffy, F. H., & Wyatt, R. J. (1983). Brain electrical activity mapping (BEAM) in schizophrenia patients. *Archives of General Psychiatry, 40,* 719-728.

Murray, R. M. & Lewis, S. W. (1987). Is schizophrenia a neurodevelopmental disorder? *British Medical Journal Clinical Research, 295,* 681-682.

Murray, R. M., O'Callaghan, E., Castle, D. J., & Lewis, S. W. (1992). A neurodevelopmental approach to the classification of schizophrenia. *Schizophrenia Bulletin, 18,* 319-332.

Naeser, M. A., Helm-Estabrooks, N., Haas, G., Auerbach, S., & Srinivasan, M. (1987). Relationship between lesion extent in 'Wernicke's area' on computed tomographic scan and predicting recovery of comprehension in Wernicke's aphasia. *Archives of Neurology, 44,* 73-82.

Nagase, Y., Okubo, Y., & Toru, M. (1996). Electroencephalography in schizophrenic patients: Comparison between neuroleptic-naive state and after treatment. *Biological Psychiatry, 40,* 452-456.

Nagy, Z., Westerberg, H., & Klingberg, T. (2005). Maturation of white matter is associated with the development of cognitive functions in childhood. *Journal of Cognitive Neuroscience, 16,* 1227-1233.

Narr, K. L., Bilder, R. M., Toga, A. W., Woods, R. P., Rex, D. E., Szeszko, P. R. et al. (2005). Mapping cortical thickness and gray matter concentration in first episode schizophrenia. *Cerebral Cortex, 15,* 708-719.

Neves-Pereira, M., Cheung, J. K., Pasdar, A., Zhang, F., Breen, G., Yates, P. et al. (2005). BDNF gene is a risk factor for schizophrenia in a Scottish population. *Molecular Psychiatry, 10,* 208-212.

Niedermeyer, E. & Lopes de Silva, F. H. (1999). *Electroencephalography: Basic principles, clinical applications and related fields*. (4th edition ed.) Baltimore: Lippincott Williams and Wilkins.

Niemann, K., Hammers, A., Coenen, V. A., Thron, A., & Klosterkotter, J. (2000). Evidence of a smaller left hippocampus and left temporal horn in both patients with first episode schizophrenia and normal control subjects. *Psychiatry Research, 99,* 93-110.

Niemi, L. T., Suvisaari, J. M., Haukka, J. K., & Lonnqvist, J. K. (2005). Childhood predictors of future psychiatric morbidity in offspring of mothers with psychotic disorder: results from the Helsinki High-Risk Study. *British Journal of Psychiatry, 186,* 108-114.

Nierenberg, J., Salisbury, D. F., Levitt, J. J., David, E. A., McCarley, R. W., & Shenton, M. E. (2005). Reduced left angular gyrus volume in first-episode schizophrenia. *American Journal of Psychiatry, 162,* 1539-1541.

Nimgaonkar, V. L. (1998). Reduced fertility in schizophrenia: here to stay? *Acta Psychiatrica Scandinavica, 98,* 348-353.

Nopoulos, P. C., Ceilley, J. W., Gailis, E. A., & Andreasen, N. C. (2001). An MRI study of midbrain morphology in patients with schizophrenia: relationship to psychosis, neuroleptics, and cerebellar neural circuitry. *Biological Psychiatry, 49,* 13-19.

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O. et al. (1997). Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET.[see comment]. *Nature, 385,* 634-636.

Okugawa, G., Sedvall, G., Nordstrom, M., Andreasen, N., Pierson, R., Magnotta, V. et al. (2002). Selective reduction of the posterior superior vermis in men with chronic schizophrenia. *Schizophrenia Research, 55,* 61-67.

Omori, M., Koshino, Y., Murata, T., Murata, I., Nishio, M., Sakamoto, K. et al. (1995). Quantitative EEG in never-treated schizophrenic patients. *Biological Psychiatry, 38,* 303-309.

Osborn, D. P. (2001). The poor physical health of people with mental illness. *Western Journal of Medicine, 175,* 329-332.

Overall, J. E. & Gorham, D. R. (1962). The Brief Psychiatric Rating Scale. *Psychological Reports, 10,* 799-812.

Paillere-Martinot, M.-L., Caclin, A., Artiges, E., Poline, J.-B., Joliot, M., Mallet, L. et al. (2001). Cerebral gray and white matter reductions and clinical correlates in patients with early onset schizophrenia. *Schizophrenia Research, 50,* 19-26.

Pakkenberg, B. (1993). Total nerve cell number in neocortex in chronic schizophrenics and controls estimated using optical disectors. *Biological Psychiatry, 34,* 768-772.

Pantelis, C., Velakoulis, D., McGorry, P. D., Wood, S. J., Suckling, J., Phillips, L. J. et al. (2003). Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet, 361,* 281-288.

Pantelis, C., Yucel, M., Wood, S. J., McGorry, P. D., & Velakoulis, D. (2003). Early and late neurodevelopmental disturbances in schizophrenia and their functional consequences. *Australian & New Zealand Journal of Psychiatry, 37,* 399-406.

Park, H. J., Levitt, J., Shenton, M. E., Salisbury, D. F., Kubicki, M., Kikinis, R. et al. (2004). An MRI study of spatial probability brain map differences between first-episode schizophrenia and normal controls. *NeuroImage, 22,* 1231-1246.

Pascual-Marqui, R. D., Michel, C. M., & Lehmann, D. (1994). Low resolution electromagnetic tomography: a new method for localizing electrical activity in the brain. *International Journal of Psychophysiology, 18,* 49-65.

Passingham, R. E. (1993). *The frontal lobes and voluntary action*. Oxford: Oxford University Press.

Paus, T., Collins, D. L., Evans, A. C., Leonard, G., Pike, B., & Zijdenbos, A. (2001). Maturation of white matter in the human brain: a review of magnetic resonance studies. *Brain Research Bulletin, 54,* 255-266.

Pearlson, G. D. & Marsh, L. (1999). Structural brain imaging in schizophrenia: a selective review. *Biological Psychiatry, 46,* 627-649.

Pekny, M. & Nilsson, M. (2005). Astrocyte activation and reactive gliosis. *Glia, 50,* 427-434.

Pfefferbaum, A., Mathalon, D. H., Sullivan, E. V., Rawles, J. M., Zipursky, R. B., & Lim, K. O. (1994). A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. *Archives of Neurology, 51,* 874-887.

Plum, F. (1972). Prospects for research on schizophrenia. 3. Neurophysiology. Neuropathological findings. *Neurosciences Research Program Bulletin, 10,* 384-388.

Purves, D. (1980). Neuronal competition. *Nature, 287,* 585-586.

Purves, D. (1998). *Body and Brain: A trophic theory of neural connections*. Cambridge, Massachusetts: Harvard University Press.

Purves, D. & Lichtman, J. W. (1980). Elimination of synapses in the developing nervous system. *Science, 210,* 153-157.

Pycock, C. J., Kerwin, R. W., & Carter, C. J. (1980). Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. *Nature, 286,* 74-76.

Raff, M. (1998). Cell suicide for beginners. *Nature, 396,* 119-122.

Raff, M. C., Barres, B. A., Burne, J. F., Coles, H. S., Ishizaki, Y., & Jacobson, M. D. (1994). Programmed cell death and the control of cell survival. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences, 345,* 265-268.

Rakic, P., Bourgeois, J. P., Eckenhoff, M. F., Zecevic, N., & Goldman-Rakic, P. S. (1986). Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science, 232,* 232-235.

Rakic, P., Bourgeois, J. P., & Goldman-Rakic, P. S. (1994). Synaptic development of the cerebral cortex: implications for learning, memory and mental illness. In J.Van Pelt, M. A. Corner, H. B. M. Uylings, & F. H. L. Da Silva (Eds.), *The self-organizing brain: from growth cones to functional networks* (102 ed., pp. 227-244). Amsterdam: Elsevier.

Rapoport, J. L., Giedd, J. N., Blumenthal, J., Hamburger, S., Jeffries, N., Fernandez, T. et al. (1999). Progressive cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. *Archives of General Psychiatry, 56,* 649-654.

Raz, S. (1993). Structural cerebral pathology in schizophrenia: regional or diffuse? *Journal of Abnormal Psychology, 102,* 445-452.

Ringo, J. L., Doty, R. W., Demeter, S., & Simard, P. Y. (1994). Time is of the essence: a conjecture that hemispheric specialization arises from interhemispheric conduction delay. *Cerebral Cortex, 4,* 331-343.

Ringuette, E. L. & Kennedy, T. (1966). An experimental study of the double bind hypothesis. *Journal of Abnormal Psychology, 71,* 136-141.

Robinson, P. A., Rennie, C. J., Wright, J. J., Bahramali, H., Gordon, E., & Rowe, D. L. (2001). Prediction of electroencephalographic spectra from neurophysiology. *Physical Review E, 63,* 021903.

Rosen, J. N. (1953). *Direct Analysis: Selected Papers*. New York: Grune and Stratton.

Sabri, O., Erkwoh, R., Schreckenberger, M., Owega, A., Sass, H., & Buell, U. (1997). Correlation of positive symptoms exclusively to hyperperfusion or hypoperfusion of cerebral cortex in never-treated schizophrenics. *Lancet, 349,* 1735-1739.

Sachdev, P. & Brodaty, H. (1999). Quantitative study of signal hyperintensities on T2-weighted magnetic resonance imaging in late-onset schizophrenia. *American Journal of Psychiatry, 156,* 1958-1967.

Saletu, B., Kufferle, B., Grunberger, J., Foldes, P., Topitz, A., & Anderer, P. (1994). Clinical, EEG mapping and psychometric studies in negative schizophrenia: comparative trials with amisulpride and fluphenazine. *Neuropsychobiology, 29,* 125-135.

Salgado-Pineda, P., Baeza, I., Perez-Gomez, M., Vendrell, P., Junque, C., Bargallo, N. et al. (2003). Sustained attention impairment correlates to gray matter decreases in first episode neuroleptic-naive schizophrenic patients. *NeuroImage, 19,* 365-75.

Salokangas, R. K., Cannon, T., Van Erp, T., Ilonen, T., Taiminen, T., Karlsson, H. et al. (2002). Structural magnetic resonance imaging in patients with first-episode schizophrenia, psychotic and severe non-psychotic depression and healthy controls. Results of the schizophrenia and affective psychoses (SAP) project. *British Journal of Psychiatry - Supplementum, 43,* s58-65.

Saykin, A. J., Shtasel, D. L., Gur, R. E., Kester, D. B., Mozley, L. H., Stafiniak, P. et al. (1994). Neuropsychological deficits in neuroleptic naive patients with first-episode schizophrenia. *Archives of General Psychiatry, 51,* 124-131.

Schaul, N. (1998). The fundamental neural mechanisms of electroencephalography. *Electroencephalography & Clinical Neurophysiology, 106,* 101-107.

Scheff, T. J. (1966). *Being mentally ill: a sociological theory*. Chicago: Aldine.

Schild, H. H. (1990). *MRI made easy (... well almost)*. Berlin: Schering AG.

Schlaepfer, T. E., Harris, G. J., Tien, A. Y., Peng, L. W., Lee, S., Federman, E. B. et al. (1994). Decreased regional cortical gray matter volume in schizophrenia. *American Journal of Psychiatry, 151,* 842-848.

Schmidt, K., Nolte-Zenker, B., Patzer, J., Bauer, M., Schmidt, L. G., & Heinz, A. (2001). Psychopathological correlates of reduced dopamine receptor sensitivity in depression, schizophrenia, and opiate and alcohol dependence. *Pharmacopsychiatry, 34,* 66-72.

Seeman, P. (1992). Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4. *Neuropsychopharmacology, 7,* 261-284.

Seeman, P., Chau-Wong, M., Tedesco, J., & Wong, K. (1975). Brain receptors for antipsychotic drugs and dopamine: direct binding assays. *Proceedings of the National Academy of Sciences of the United States of America, 72,* 4376-4380.

Seeman, P. & Kapur, S. (2000). Schizophrenia: more dopamine, more D2 receptors. *Proceedings of the National Academy of Sciences of the United States of America, 97,* 7673-7675.

Seeman, P., Lee, T., Chau-Wong, M., & Wong, K. (1976). Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature, 261,* 717-719.

Selemon, L. D. & Goldman-Rakic, P. S. (1999). The reduced neuropil hypothesis: A circuit based model of schizophrenia. *Biological Psychiatry, 45,* 17-25.

Selemon, L. D., Rajkowska, G., & Goldman-Rakic, P. S. (1998). Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. *Journal of Comparative Neurology, 392,* 402-412.

Sevy, S. & Davidson, M. (1995). The cost of cognitive impairment in schizophrenia. *Schizophrenia Research, 17,* 1-3.

Shapleske, J., Rossell, S. L., Chitnis, X. A., Suckling, J., Simmons, A., Bullmore, E. T. et al. (2002). A computational morphometric MRI study of schizophrenia: effects of hallucinations. *Cerebral Cortex, 12,* 1331-41.

Sharma, T. & Chitnis, X. (2000). *Brain Imaging in Schizophrenia: Insights and Applications*. London: ReMEDICA Publishing Ltd.

Shelton, R. C. & Weinberger, D. R. (1986). X-ray computerized tomography studies in schizophrenia: a review and synthesis. In H.A.Nasrallah & D. R. Weinberger (Eds.), *Handbook of Schizophrenia: the Neuropathology of Schizophrenia, vol.I.* (pp. 207-225). New York: Elsevier Science.

Shenton, M. E., Dickey, C. C., Frumin, M., & McCarley, R. W. (2001). A review of MRI findings in schizophrenia. *Schizophrenia Research, 49,* 1-52.

Shergill, S. S., Brammer, M. J., Fukuda, M., Williams, S. C., Murray, R. M., & McGuire, P. K. (2003). Engagement of brain areas implicated in processing inner speech in people with auditory hallucinations. *British Journal of Psychiatry, 182,* 525-531.

Shergill, S. S., Brammer, M. J., Williams, S. C., Murray, R. M., & McGuire, P. K. (2000). Mapping auditory hallucinations in schizophrenia using functional magnetic resonance imaging. *Archives of General Psychiatry, 57,* 1033-1038.

Shoval, G. & Weizman, A. (2005). The possible role of neurotrophins in the pathogenesis and therapy of schizophrenia. *European Neuropsychopharmacology, 15,* 319-329.

Sigmundsson, T., Suckling, J., Maier, M., Williams, S., Bullmore, E., Greenwood, K. et al. (2001). Structural abnormalities in frontal, temporal and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. *American Journal of Psychiatry, 158,* 234-243.

Silverstein, S. M., Kovacs, I., Corry, R., & Valone, C. (2000). Perceptual organization, the disorganization syndrome, and context processing in chronic schizophrenia. *Schizophrenia Research, 43,* 11-20.

Siris, S. (1995). Depression and schizophrenia. In S.R.Hirsch & D. R. Weinberger (Eds.), *Schizophrenia* (pp. 128-145). Oxford: Blackwell Science.

Sisk, C. L. & Foster, D. L. (2004). The neural basis of puberty and adolescence. *Nature Neuroscience, 7,* 1040-1047.

Southard, E. E. (1914). On the topographical distribution of cortex lesions and anomalies in dementia precox, with some account of their functional significance. *American Journal of Insanity, 71,* 383-405.

Sowell, E. R., Thompson, P. M., Holmes, C. J., Batth, R., Jernigan, T. L., & Toga, A. W. (1999). Localizing age-related changes in brain structure between childhood and adolescence using statistical parametric mapping. *NeuroImage, 9,* 587-597.

Spence, S. A., Brooks, D. J., Hirsch, S. R., Liddle, P. F., Meehan, J., & Grasby, P. M. (1997). A PET study of voluntary movement in schizophrenic patients experiencing passivity phenomena (delusions of alien control). *Brain, 120,* 1997-2011.

Sponheim, S. R., Clementz, B. A., Iacono, W. G., & Beiser, M. (2000). Clinical and biological concomitants of resting state EEG power abnormalities in schizophrenia. *Biological Psychiatry, 48,* 1088-1097.

Srole, L., Langner, T. S., Michael, S. T., Opler, M. K., & Rennie, T. A. C. (1962). *Mental health in the metropolis: the mid-town Manhattan study*. New York: McGraw-Hill.

Steen, R. G., Ogg, R. J., Reddick, W. E., & Kingsley, P. B. (1997). Age-related changes in the pediatric brain: quantitative MR evidence of maturational changes during adolescence. *American Journal of Neuroradiology, 18,* 819-828.

Sullivan, H. S. (1924). Schizophrenia: Its Conservative and Malignant Features. A Preliminary Communication. *American Journal of Psychiatry, 4,* 77-91.

Susser, E., Neugebauer, R., Hoek, H. W., Brown, A. S., Lin, S., Labovitz, D. et al. (1996). Schizophrenia after prenatal famine. Further evidence. *Archives of General Psychiatry, 53,* 25-31.

Szekeres, G., Juhasz, A., Rimanoczy, A., Keri, S., & Janka, Z. (2003). The C270T polymorphism of the brain-derived neurotrophic factor gene is associated with schizophrenia. *Schizophrenia Research, 65,* 15-18.

Szeszko, P. R., Ardekani, B. A., Ashtari, M., Kumra, S., Robinson, D. G., Sevy, S. et al. (2005). White matter abnormalities in first-episode schizophrenia or schizoaffective disorder: a diffusion tensor imaging study. *American Journal of Psychiatry, 162,* 602-605.

Talairach, J. & Tournoux, P. (1988). *Co Planar Steroetaxic Atlas of the Human Brain*. New York: Thieme Medical Publishers.

Thara, R. & Srinivasan, T. N. (1997). Outcome of marriage in schizophrenia. *Social Psychiatry & Psychiatric Epidemiology, 32,* 416-420.

Thatcher, R. W., Biver, C., McAlaster, R., Camacho, M., & Salazar, A. (1998). Biophysical linkage between MRI and EEG amplitude in closed head injury. *NeuroImage, 7,* 352-367.

Thompson, P. M., Vidal, C., Giedd, J. N., Gochman, P., Blumenthal, J., Nicholson, R. et al. (2001). Mapping adolescent brain change reveals dynamic wave of accelerated grey matter loss in very early-onset schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America, 98,* 11650-11655.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N. et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage, 15,* 273-289.

Valente, A. A., Jr., Miguel, E. C., Castro, C. C., Amaro E Jr, Duran, F. L., Buchpiguel, C. A. et al. (2005). Regional gray matter abnormalities in obsessive-compulsive disorder: a voxel-based morphometry study. *Biological Psychiatry, 58,* 479-487.

Van Rossum, J. (2006). The significance of dopamine-receptor blockade for the action of neuroleptic drugs. In H.Brill, J. Cole, P. Deniker, H. Hippius, & P. Bradley (Eds.), *Neuropsychopharmacology, Proceedings Fifth Collegium Internationale Neuropsychopharmacologicum* (pp. 321-329). Amsterdam: Excerpta Medica.

Velakoulis, D., Pantelis, C., McGorry, P. D., Dudgeon, P., Brewer, W., Cook, M. et al. (1999). Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. *Archives of General Psychiatry, 56,* 133-141.

Velakoulis, D., Wood, S. J., Smith, D. J., Soulsby, B., Brewer, W., Leeton, L. et al. (2002). Increased duration of illness is associated with reduced volume in right medial temporal/anterior cingulate grey matter in patients with chronic schizophrenia. *Schizophrenia Research, 57,* 43-49.

Vita, A., Dieci, M., Giobbio, G. M., Tenconi, F., & Invernizzi, G. (1997). Time course of cerebral ventricular enlargement in schizophrenia supports the hypothesis of its neurodevelopmental nature. *Schizophrenia Research, 23,* 25-30.

Wechsler, D. (1997). *Wechsler Abbreviated Scale of Intelligence (WASI) manual*. San Antonio: Psychological Corporation.

Weinberger, D. R. (1987). Implications of normal brain development for the pathogenesis of schizophrenia. *Archives of General Psychiatry, 44,* 660-669.

Whalley, H. C., Simonotto, E., Marshall, I., Owens, D. G., Goddard, N. H., Johnstone, E. C. et al. (2005). Functional disconnectivity in subjects at high genetic risk of schizophrenia. *Brain, 128,* 2097-2108.

Whitford, T. J., Farrow, T. F., Gomes, L., Brennan, J., Harris, A. W., & Williams, L. M. (2005). Grey matter deficits and symptom profile in first episode schizophrenia. *Psychiatry Research - Neuroimaging, 139,* 229-238.

Whitford, T. J., Farrow, T. F., Rennie, C. J., Grieve, S. M., Gomes, L., Brennan, J. et al. (2007a). Longitudinal changes in neuroanatomy and neural activity in early schizophrenia. *NeuroReport, 18,* 435-439.

Whitford, T. J., Grieve, S. M., Farrow, T. F., Gomes, L., Brennan, J., Harris, A. W. F. et al. (2006). Progressive grey matter atrophy over the first 2-3 years of illness in first-episode schizophrenia: a tensor-based morphometry study. *NeuroImage, 32,* 511-519.

Whitford, T. J., Grieve, S. M., Farrow, T. F., Gomes, L., Brennan, J., Harris, A. W. F. et al. (2007b). Volumetric white matter abnormalities in first-episode schizophrenia: a longitudinal, tensor-based morphometry study. *American Journal of Psychiatry, 164,* 1082-1089.

Whitford, T. J., Rennie, C. J., Grieve, S. M., Clark, C. R., Gordon, E., & Williams, L. M. (2007c). Brain maturation in adolescence: concurrent changes in neuroanatomy and neurophysiology. *Human Brain Mapping, 28,* 228-237.

Whitworth, A. B., Kemmler, G., Honeder, M., Kremser, C., Felber, S., Hausmann, A. et al. (2005). Longitudinal volumetric MRI study in first- and multiple-episode male schizophrenia patients. *Psychiatry Research, 140,* 225-237.

Wible, C. G., Shenton, M. E., Hokama, H., Kikinis, R., Jolesz, F. A., Metcalf, D. et al. (1995). Prefrontal cortex and schizophrenia. A quantitative magnetic resonance imaging study. *Archives of General Psychiatry, 52,* 279-288.

Wilkinson, G. S. (1993). *The Wide Range Achievement Test - Administration Manual*. Wilmingham: Wide Range Inc.

Williams, R. W. & Herrup, K. (1988). The control of neuron number. *Annual Review of Neuroscience, 11,* 423-453.

Worsley, K. J., Evans, A. C., Marrett, S., & Neelin, P. (1992). A three-dimensional statistical analysis for CBF activation studies in human brain. *Journal of Cerebral Blood Flow & Metabolism, 12,* 900-918.

Worsley, K. J., Marrett, S., Neelin, P., Vandal, A. C., Friston, K. J., & Evans, A. C. (1996). A unified statistical approach for determining significant signals in images of cerebral activation. *Human Brain Mapping, 4,* 58-73.

Wright, I. C., McGuire, P. K., Poline, J.-B., Travere, J. M., Murray, R. M., Frith, C. D. et al. (1995). A voxel-based method for the statistical analysis of gray and white matter density applied to schizophrenia. *NeuroImage, 2,* 224-252.

Yakovlev, P. & Lecours, A. (1967). The myelinogenetic cycles of regional maturation of the brain. In A.Minkowski (Ed.), *Regional development of the brain early in life* (pp. 3-70). Boston, Massachusetts: Blackwell Scientific Publications.

Zhang, F., Sarginson, J., Crombie, C., Walker, N., St Clair, D., & Shaw, D. (2006). Genetic association between schizophrenia and the DISC1 gene in the Scottish population. *American Journal of Medical Genetics.Part B, Neuropsychiatric Genetics: the Official Publication of the International Society of Psychiatric Genetics, 141,* 155-159.

Zipursky, R. B., Marsh, L., Lim, K. O., DeMent, S., Shear, P. K., Sullivan, E. V. et al. (1994). Volumetric MRI assessment of temporal lobe structures in schizophrenia. *Biological Psychiatry, 35,* 501-516.