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Exploring the Neural Substrates Contributing to the Risk for Psychosis: An MRI Investigation

By,

Sarah Jacobson

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy to the University of Dublin, Trinity College

Supervisor: Professor Hugh Garavan

Department of Psychology

March 2010
Declaration

I hereby declare that this thesis, submitted to the University of Dublin, Trinity College, is entirely my own work, unless otherwise stated, and that it has not been previously submitted to this or any other university. I give my permission to the library to lend or photocopy this thesis upon request.

Date

8/3/10
Acknowledgements

The tale of the Ph.D.

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Summary

Over the last three decades schizophrenia research has shifted its focus from studying individuals with the established illness to those who have an elevated risk for developing the disease. Research has suggested that schizophrenia is a neurodevelopmental disorder which may be negatively affected by genetic and environmental insults which may contribute to the neurobiological abnormalities and cognitive deficits observed in schizophrenia patients. One etiological approach to improving our understanding of the pathophysiology of the disease is to study the developing brains of children who are experiencing subclinical psychotic symptoms. In this thesis I investigate neurocognition, brain structure (VBM and DTI FA), function (fMRI), and resting-state functional connectivity in children with an increased vulnerability for developing psychosis, due to the presence of psychotic-like symptoms, through the use of non-invasive multi-modal MRI techniques.

Chapters Two, Three and Four explored the neural correlates of the vulnerability stage of psychosis through investigating children who are experiencing psychotic-like symptoms. Chapter Two describes how the at-risk children were identified, which was through a population-based study of schoolchildren, aged 11-13 years old, who were experiencing subclinical psychotic symptoms. Control and at-risk children completed a multi-modal MRI investigation. Functional deficits during a response inhibition task were found in right prefrontal and middle temporal cortices. Volumetric increases in grey matter were found in the at-risk children in prefrontal, temporal and parietal regions. White matter integrity revealed reductions in the AR children in the parahippocampus, temporal, and occipital regions.

Chapter Three examined a group of chronic schizophrenia patients using the same multi-modal MRI measures. Patients revealed right prefrontal, cingulate and
subcortical functional reductions. Increased grey matter in the patient group was
found in temporal and parietal regions. Decreased FA in the schizophrenia patients
was localised along long association fibres in frontal, temporal, cingulate and
cerebellar regions. I conducted a combined group analysis to compare the two
psychosis groups and the two control groups. This analysis revealed reduced activity
in the right prefrontal, temporal, and claustrum in the psychosis groups. Using a
similar method to explore differences in regional brain volumes between groups a
conjunction analysis was carried out and identified reduced grey matter in the right
insula in the psychosis groups compared to the control groups. The FA analysis
revealed decreased white matter integrity in the psychosis groups in the
parahippocampus, ventral striatum, and parietal lobe.

Chapter Four examined the inter- and intra-hemispheric connectivity
disturbances, which has been suggested to play a major role in the development of
schizophrenia. Seed regions were chosen from the fMRI and VBM results previously
reported. The psychosis groups revealed reduced connectivity in predominately the
right hemisphere, in frontal, parietal, cingulate, and striatal brain areas.

The functional and structural abnormalities found in common in the psychosis
groups may reveal early biomarkers related not only to the vulnerability for
developing psychosis but could be persistent and for the basis of the underlying
aberrant neuropathology present in those who have developed the disease.
### Abbreviations

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<td>ACC</td>
<td>anterior cingulate cortex</td>
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<tr>
<td>AFNI</td>
<td>Analysis of Functioning Neuroimages</td>
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<tr>
<td>APSS</td>
<td>Adolescent Psychotic-like Symptoms Scale</td>
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<tr>
<td>ARMS</td>
<td>at-risk mental state</td>
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<tr>
<td>AVH</td>
<td>auditory-verbal hallucinations</td>
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<tr>
<td>BA</td>
<td>Broadmann Area</td>
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<tr>
<td>BPRS</td>
<td>Brief Psychiatric Rating Scale</td>
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<tr>
<td>BOLD</td>
<td>blood oxygen level dependent</td>
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<tr>
<td>COMT</td>
<td>cathechol-o-methyltransferase</td>
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<tr>
<td>CPT</td>
<td>Continuous performance test</td>
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<tr>
<td>DA</td>
<td>dopamine</td>
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<tr>
<td>DLPFC</td>
<td>dorsal lateral prefrontal cortex</td>
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<tr>
<td>DMN</td>
<td>default-mode network</td>
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<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>EEG</td>
<td>electroencephalogram</td>
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<tr>
<td>EHRS</td>
<td>Edinburgh High-Risk Study</td>
</tr>
<tr>
<td>EPI</td>
<td>echo-planar pulse imaging</td>
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<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>FWHM</td>
<td>full width half-max</td>
</tr>
<tr>
<td>GM</td>
<td>grey matter</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IFG</td>
<td>inferior frontal gyrus</td>
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<tr>
<td>KSADS</td>
<td>Schedule for Affective Disorders and Schizophrenia for School-Aged Children</td>
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<tr>
<td>MPRAGE</td>
<td>magnetisation prepared rapid gradient echo</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>NAcc</td>
<td>nucleus accumbens</td>
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<tr>
<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>RSFC</td>
<td>resting-state functional connectivity</td>
</tr>
<tr>
<td>RT</td>
<td>reaction time</td>
</tr>
<tr>
<td>SART</td>
<td>Sustained Attention to Response Test</td>
</tr>
<tr>
<td>SFG</td>
<td>superior front gyrus</td>
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<tr>
<td>SIPS</td>
<td>Structured Interview of Prodromal Symptoms</td>
</tr>
<tr>
<td>SLF</td>
<td>superior longitudinal fasciculus</td>
</tr>
<tr>
<td>SMA</td>
<td>supplementary motor area</td>
</tr>
<tr>
<td>STOPS</td>
<td>successful inhibitions</td>
</tr>
<tr>
<td>STG</td>
<td>superior temporal gyrus</td>
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<tr>
<td>TMT</td>
<td>Trail-making test</td>
</tr>
<tr>
<td>TR</td>
<td>time of repetition</td>
</tr>
<tr>
<td>UF</td>
<td>uncinate fasciculus</td>
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<tr>
<td>UHR</td>
<td>ultra-high risk</td>
</tr>
<tr>
<td>VBM</td>
<td>voxel-based morphometry</td>
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<td>VLPFC</td>
<td>ventral lateral prefrontal cortex</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>WCST</td>
<td>Wisconsin Card Sort Task</td>
</tr>
<tr>
<td>WM</td>
<td>white matter</td>
</tr>
<tr>
<td>%AUC</td>
<td>percentage area under the curve</td>
</tr>
<tr>
<td>%CS</td>
<td>percentage change score</td>
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1. Chapter One
An introduction to the risk for psychosis
1.1 What is schizophrenia

The etymology of the word schizophrenia, which was first coined by Bleuler (1911), reveals the disturbing essence of this disease: a split mind. At the core of this debilitating mental illness lie cognitive disturbances which disrupt the entire neural network within the brain (Bleuler, 1911; Kraepelin, 1919), giving rise to a cacophony of disorganised and irrational thinking. Schizophrenia, as defined in the Diagnostic and Statistical Manual of Mental Disorders 4th Edition-Revised (DSM-IV) (APA, 2000), requires the all three following diagnostic criteria to be met: Characteristic symptoms, social or occupational dysfunction and duration. Characteristic symptoms include hallucinations, delusions, disorganised speech or behaviour, and negative symptoms, of which two must be present for the majority of the time during a one-month period. Social or occupational dysfunction refers to a marked deterioration in functioning, in such areas as work, school, relationships, personal care, that have become pervasive since the onset of the disturbance. The duration criteria states that the presence of the disturbances must be continuously present for at least six months, including the one-month period of characteristic symptoms.

It is generally accepted that the presence of psychotic symptoms are derived from aberrant developmental trajectories (Jones, Rodgers, Murray, & Marmot, 1994; Marenco & Weinberger, 2000). In this thesis specific attention will be paid to abnormalities presenting in the beginning of the second decade of life. Investigations of schizophrenia during childhood development (i.e. pre-adolescence) are intended to be retoprospective, as the formal diagnostic symptoms of schizophrenia do not normally manifest and reach clinical intervention until late adolescence or young adulthood (i.e. ages 18-35 years) (Jobe & Harrow, 2005). Although schizophrenia presents itself in a heterogeneous form in each unique individual diagnosed with a
psychotic mental illness, there are three basic symptom classifications: positive, negative, and disorganised symptoms, which are commonly expressed during the course of the disease.

The formal diagnostic criteria of schizophrenia has been clearly outlined above; however, studies of the premorbid symptoms of the illness, for example, hallucinations not meeting the duration criteria for schizophrenia or the presence of genetic trait markers for the disease, have not yet yielded the specify necessary to differentiate the conversion to schizophrenia from other possible clinical outcomes, such as, schizoaffective disorder, bipolar disorder, anxiety and other mood disorders, during the risk stage. One study which will be commonly referred to in this thesis is Poulton et al. (2000), whose description of potential premorbid symptoms form the basis of the at-risk study described in this thesis, revealed increased sensitivity to the specific subsequent diagnosis of schizophrenia. The term “psychosis” in this thesis will hitherto refer to individuals who are at an elevated risk for developing schizophrenia and other related mental illness. To clarify, due to the lack of conclusive evidence that individuals with subclinical symptoms will only or possibly in conjunction with develop schizophrenia and not one of the other aforementioned clinical outcomes, this term will be used when referring to the risk phase instead of “schizophrenia.”

1.1.1 Clinical symptomotology

Positive symptoms

The hallmark symptoms of established schizophrenia are the positive symptoms, such as delusions (fixed, false beliefs) and hallucinations (aberrant perceptual experiences). Delusions are false beliefs that are held with a strong conviction despite a lack supporting evidence. Delusions are most commonly
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characterised as paranoid, as patients commonly report the heavily persecutory content of their delusional experiences. A core process reinforced in this belief system is that negative interactions will be experienced in the future (Bentall, et al., 2008).

Physiological data from neuroimaging investigations reveal that auditory-verbal hallucinations (AVH) are accompanied by activations in speech monitoring and production regions (McGuire, Shah, & Murray, 1993). This research has suggested that AVHs are a result of the misattribution of inner speech to an external source (Bentall, 1990; Hoffman, Stopek, & Andreasen, 1986), leading to the misperception of hearing a voice or sound. It has been reported that between 60-80% of schizophrenia patients suffer from AVH (Andreasen & Flaum, 1991; Stephane, Barton, & Boutros, 2001). Positive symptoms, such as AVH, commonly give rise to impaired functioning in the schizophrenia patients due to the intrusive nature of these positive symptoms.

Since positive symptoms can be readily identified and quantified through clinical assessments, such as the Structured Clinical Interview for DSM Disorders (SCID) (First, Spitzer, Gibbon, & Williams, 2002) and the Structured Interview for Prodromal Symptoms (SIPS) (McGlashan, Miller, Woods, Hoffman, & Davidson, 2001) these symptoms can serve as the earliest identifiable clinical manifestations of the development to a diagnosis of schizophrenia.

Negative symptoms

A different profile of behavioural disturbances is evident in the negative symptoms experienced in schizophrenia patients. Negative symptoms are appropriately named as these are behaviours that are normally present in healthy individuals but lacking in patients with schizophrenia. Common negative symptoms
include flattened affect, alogia, avolition and apathy (APA, 2000). Flattened, or blunted, affect is characterised by the reduced ability to express emotional valences. Patients with a flattened affect usually present as unresponsive and expressionless, even when conversation is directed to them. The negative symptom of alogia, is also referred to as impoverished speech, and reflects the patient’s attempt to cope with this sensory overload, therefore the patient does not initiate conversations or produces speech that is devoid of content. Avolition is known as the poverty of will, and is an inability to initiate and complete goal-directed behaviours. This symptom is normally coupled with apathy, which is characterised by a lack of interest or concern in partaking in social, emotional, or physical activities or behaviours. Neuroimaging studies which assess social cognition in schizophrenia seek to isolate the neural underpinnings of these negative symptoms.

**Disorganised symptoms**

The cluster of symptoms known as disorganised symptoms also encompasses the cognitive symptoms of schizophrenia, which underlie the disorganised behavioural manifestation which results from aberrant neural pathology, in turn affecting the cognitive abilities of patients resulting in the negative symptoms. Cognitive deficits in schizophrenia have been shown in various laboratory studies which clearly indicate that patients perform poorly on information processing and perceptual tasks. According to some researchers the core deficit seems to be a breakdown in the relationship between stored information and current sensory input (Glahn, et al., 2005; Niedman, et al., 2006). Delusions and hallucinations are thought to arise from sensory overload and the inability to filter. This in turn impairs logical thinking thus rendering this population prone to make more mistakes on such laboratory tasks. Therefore, the culmination of the development of a psychotic illness
can be attributed to the underlying disruption in orchestrating basic cognitive processes. Assessment of executive functioning, connectivity and underlying structural abnormalities will be investigated in the following chapters to explore the neurobiological underpinnings of the vulnerability to developing schizophrenia.

Formal thought disorder is a sub-classification of disorganised symptoms. Schizophrenia has been described as a disturbance in the form and content of normal thinking which disrupts the flow of goal-directed behaviours (Drakeford, et al., 2006). Andreasen (1979) expanded on the clinical description of formal thought disorder which was originally defined by Taylor et al. (1981). Andreasen identified the following categories related to abnormalities in speech production: poverty of speech, poverty of content of speech, pressure of speech, distractible speech, incoherence, circumscriptionality, echolalia, and blocking. These symptoms are presumed to reflect a disruption in the flow of conscious verbal thought that is inferred from spoken language. Andreasen et al. (1998) proposed a single phenotype exits which explained the core cognitive deficits of schizophrenia which were neurodevelopmental in origin. They referred to this phenotype as “cognitive dysmetria” which was defined as a difficulty in prioritizing, processing, coordinating and responding to information.

Evidence for a disruption in cognitive abilities in schizophrenia patients have been identified in numerous behavioural and functional neuroimaging studies and are described in a later section of this chapter.

1.1.2 First-episode of schizophrenia

Descriptions of the first-episode of a major psychotic experience have been heterogeneous and include many dimensions of behavioural and cognitive functioning which are impaired at the time of onset. Therefore, the first-episode is not normally the first instance of a disruption in behavioural or cognitive processing, but rather a
culmination of a number of symptoms which result in a major “break” in normal functioning. First-episode psychotic patients normally do not seek hospital or psychiatric services until after a significant amount of time of experiencing psychotic symptoms has lapsed, averaging 12-24 months (Fenton, Blyler, & Heinssen, 1997; Gift, Strauss, Harder, Kokes, & Ritzler, 1981).

Keshavan and Schooler (1992) have identified six clinical events which must be examined to identify the onset of first-episode psychosis. These include: decline in social functioning, onset of general behavioural problems, onset of positive symptoms, onset of negative symptoms, first hospital admission, and first treatment. McGorry et al. (2006) uses a clinical staging model, which is a well-established model in the treatment of general medical conditions, to describe the transition stages leading to the development of a psychotic illness (Table 1.1). In the first state patient’s positive symptoms, such as delusions, are coupled with the experience of beginning to feel a loss of control over their cognitive processes, but only cause mild or moderate impairments in functioning. They may also experience a decline in functioning and feelings of low mood and social withdrawal. This is usually followed by stage two, the first-episode, which is when the severity of symptoms and cognitive and global functioning require clinical intervention. After the end of the first-episode, in stage two, social disability and the objective social status normally remain fairly stable over a period of at least three years in patients who respond to treatment and rehabilitation. Stage three emphases the response to clinical interventions, where remission of symptoms may occur, or a relapse of residual symptoms, which lead to decreased global functioning. In stage four psychotic thinking persists causing severe impairments in neurocognition and behavioural functioning.
### Table 1.1

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>Definition</th>
<th>Target populations for recruitment</th>
<th>Potential interventions</th>
<th>Indicative biological and endophenotypic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Increased risk of psychotic disorder No symptoms currently</td>
<td>First degree teenage relatives of probands</td>
<td>Improved mental health literacy, family education, drug education, brief cognitive skills training</td>
<td>Trait marker candidates and endophenotypes, e.g., smooth pursuit eye movements, P50, niacin sensitivity, mismatch negativity, olfactory deficits, etc.</td>
</tr>
<tr>
<td>1a</td>
<td>Mild or non-specific symptoms, including neurocognitive deficits, mild functional change or decline</td>
<td>Screening of teenage populations, referral by primary care physicians, referral by school counselors</td>
<td>Formal mental health literacy, family psychoeducation, formal cognitive—behavioural therapy, active substance misuse reduction</td>
<td>Trait and state candidates where feasible according to sample size</td>
</tr>
<tr>
<td>1b</td>
<td>Ultra-high risk: moderate but subthreshold symptoms, with moderate neurocognitive changes and functional decline to caseness</td>
<td>Referral by educational agencies, primary care physicians, emergency departments, welfare agencies</td>
<td>Family psychoeducation, formal cognitive—behavioural therapy, active substance misuse reduction</td>
<td>Niacin sensitivity, folate status, magnetic resonance imaging and magnetic resonance spectroscopy changes, hypothalamic—pituitary—adrenal axis dysregulation</td>
</tr>
<tr>
<td>2</td>
<td>First episode of psychotic disorder Full threshold disorder with moderate—severe symptoms, neurocognitive deficits and functional decline (Global Assessment of Functioning (GAF) 30–50)</td>
<td>Referral by primary care physicians, emergency departments, welfare agencies, specialist care agencies, drug and alcohol services</td>
<td>Family psychoeducation, formal cognitive—behavioural therapy, active substance misuse reduction, atypical antipsychotic agents for episode rehabilitation</td>
<td>Continue with markers of illness state, trait and progression</td>
</tr>
<tr>
<td>3a</td>
<td>Incomplete remission from first episode of care Could be linked or fast-tracked to stage 4</td>
<td>Primary and specialist care services</td>
<td>As for stage 2 with additional emphasis on medical and psychosocial strategies to achieve full remission</td>
<td>Continue with markers of illness state, trait and progression</td>
</tr>
<tr>
<td>3b</td>
<td>Recurrence or relapse of psychotic disorder which stabilises with treatment at a level of GAF, residual symptoms, or neurocognitive below the best level achieved following remission from first episode</td>
<td>Primary and specialist care services</td>
<td>As for stage 3a with additional emphasis on relapse prevention and ‘early warning signs’ strategies</td>
<td>Continue with markers of illness state, trait and progression</td>
</tr>
<tr>
<td>3c</td>
<td>Multiple relapses, provided worsening in clinical extent and impact of illness is objectively present</td>
<td>Specialist care services</td>
<td>As for stage 3b with emphasis on long-term stabilisation</td>
<td>Continue with markers of illness state, trait and progression</td>
</tr>
<tr>
<td>4</td>
<td>Severe, persistent or unremitting illness as judged on symptoms, neurocognitive and disability criteria. Note: could fast track to this stage at first presentation through specific clinical and functional criteria (from stage 2) or alternatively by failure to respond to treatment (from stage 3a)</td>
<td>Specialised care services</td>
<td>As for stage 3c but with emphasis on clozapine, other tertiary treatments, social participation despite ongoing disability</td>
<td>Continue with markers of illness state, trait and progression</td>
</tr>
</tbody>
</table>

### 1.2 Epidemiology of schizophrenia

In an epidemiological study (Häfner & Nowotny, 1995) gathered a sample of 232 first-episode of schizophrenia patients. These researchers retrospectively assessed the first signs and symptoms of the disorder and course of their illness. They conducted a follow-up study of a representative subgroup based on the findings of the first study (n= 133). This subgroup was prospectively examined in five time-points
over three years from their first admission on. This study examined the age of onset relative to clinical outcome. They examined early-onset schizophrenia patients (aged ≤ 20 years), medium-onset group (21 - 35 years) and a late-onset group (35 - ≤ 60 years) with regard to age and type of onset, early symptom-related course, social development and social course. Although the number of schizophrenia-specific positive and negative symptoms in early-onset schizophrenia is comparable to that of higher age groups, neurotic symptoms, emotional disorders and conduct disorders are most frequent in younger patients, especially in young men. An earlier onset of schizophrenia has more severe social consequences than onset in adults, because it interrupts cognitive and social development at an earlier stage in life. Therefore, a poorer social outcome in schizophrenia is not necessarily related to more severe symptomatology, but to the earlier age at onset and the impairment or stagnation of social ascent at an earlier stage of social and cognitive development.

Statistical research into the incidence of schizophrenia has revealed that the median incidence of schizophrenia is estimated to occur in 15.2/100,000 persons per year, with the central 80% of estimates significantly varied (7.7-43.0/100,000). Additionally the average person has around a 0.7% (7.2/1,000) chance of developing psychosis during their lifetime (McGrath, Saha, Chant, & Welham, 2008). A recent systematic review of the epidemiology of schizophrenia highlighted the prevalence of schizophrenia through demographic risk factors which may influence the development of the illness (McGrath & Susser, 2009). In summary, they found risk factors associated with the illness included being a male (M:F, 1.4:1), being a migrant vs. a native-born resident, living in an urban area vs. mixed urban/rural settings, living in a developing nation, and residing in a more extreme northern or southern latitude.
However, these demographic risk factors alone cannot explain in full the vulnerability factors which lead to the development of psychosis.

1.3 Theories of schizophrenia

Two dimensions which contribute to the neural disturbances associated with schizophrenia: genetic-environmental risk factors and early-to-late maturational causes falling along an expression of continuum. The dominant theory of schizophrenia is the neurodevelopmental model. Within the framework of this model individual risk factors can be identified at specific time points allowing the identification of genetic and environmental risk factors during a given window of time. Currently, the examination of neurodevelopment during the premorbid phase is gaining critical mass as this period is free of medication effects and illness chronicity that are inherent in studies of patients with the established illness. Longitudinally tracking neurodevelopmental changes in vivo beginning from conception to the onset of the illness in patients would provide a full picture of the neurodevelopmental course of the disease; however, these studies have yet to be carried out. However, research using neurodevelopmental models do provide cross-sectional snapshots of the disease progression at different stages.

Another theory of schizophrenia which stems from the neurodevelopmental model and delves deeper into the neurobiological factors contributing to the expression of the disease is the dopamine hypothesis of schizophrenia. Research into this theory has revealed that altered gene expression during late-neurodevelopment causes variations in mesolimbic dopamine (DA) expression, leading to misattribution of salience to endogenous and exogenous stimuli (Howes & Kapur, 2009).

The final theory, which will be described, is the disconnection hypothesis of schizophrenia. The disconnection hypothesis states that a disruption occurring in
neural connectivity adversely affects the structure of neurocognitive networks in the brain (Bullmore, Frangou, & Murray, 1997; Friston, 1998; Friston & Frith, 1995). This theory falls closely to the original observations of disrupted neural networks discovered almost 80 years previously by Bleuler (1911) and Kraepelin (1919). However, modern in vivo neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI), and resting-state functional connectivity (RSFC) analyses have helped to elucidate specific regions of abnormal neural integration during the course of schizophrenia.

1.3.1 Neurodevelopmental model

The neurodevelopmental theory of schizophrenia states that schizophrenia is the behavioural outcome of an aberration occurring during the brain developmental process, which may be caused by a combination of environmental and genetic factors (Singh, McDonald, Murphy, & O'Reilly, 2004). Marenco and Weinberger (2000) conducted a review of the literature related to the neurodevelopmental hypothesis and concluded that schizophrenia was indeed not a purely neurodegenerative disease, as revealed by the stability in brain structure over time and the absence of post-mortem cortical degeneration, but rather arises from a brain insult at or around birth. A substantial body of work examining early environmental risk factors has revealed that a considerable number of individuals who later develop schizophrenia have experienced a prenatal insult; some examples include maternal infections during pregnancy (Clarke, Tanskanen, Huttunen, Whittaker, & Cannon, 2009; Jones, Rantakallio, Hartikainen, Isohanni, & Sipila, 1998; Shi, Fatemi, Sidwell, & Patterson, 2003), complications of pregnancy (i.e. bleeding or diabetes) and abnormal foetal development (i.e. congenital malformations) (see meta-analysis in Cannon, Jones, & Murray, 2002). Additional early risk factors include perinatal obstetric complications.
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(Cannon, Huttunen, Tanskanen, Arseneault, Jones, & Murray, 2002) specifically foetal hypoxia (Cannon, et al., 2003; Van Erp, et al., 2002), which been estimated to occur in 2% of those who later develop schizophrenia (Geddes, et al., 1999). Figure 1.1 summarises the neurodevelopmental risk factors associated with schizophrenia in relation to time. Longitudinal investigations of childhood development, which have studied individuals who later go on to develop schizophrenia, show motor, language, social-affective delays, and cognitive impairments during infancy (Bearden, Rosso, Hollister, Sanchez, Hadley, & Cannon, 2000; Cannon, Huttunen, Tanskanen, Arseneault, Jones, & Murray, 2002; Cannon, et al., 2003; Jones, Rodgers, Murray, & Marmot, 1994).

Figure 1.1. Neurodevelopmental hypothesis of schizophrenia. Two independent dimensions of neural disturbances in schizophrenia, with examples of relevant factors. Adapted from Cannon et al. (2003).

A study of patients with early-onset schizophrenia (defined as onset of psychosis by age 12) (Nicolson & Rapoport, 1999) revealed that higher rates of
familial psychotic disorders, higher instance of cytogenetic anomalies, and more severe premorbid neurodevelopmental abnormalities are all etiological risk factors associated with childhood-onset schizophrenia. Asarnow et al. (1994) found that individuals with childhood-onset schizophrenia tend to have poorer outcomes than those with the onset of the illness in late adolescence or adulthood. Such evidence would suggest that an early onset of schizophrenia would lead to slightly different and a more debilitating trajectory of the disease which was present, at least in part, from very early in life, mediated by greater familial vulnerability, and is related to a more severe expression of the illness.

Risk factors emerging in late-neurodevelopment occur in childhood and have been associated with such events as childhood trauma (Kelleher, et al., 2008) and sexual abuse (Arseneault, Cannon, Witton, & Murray, 2004). Cannabis abuse is a recently identified risk factor which co-occurs with late-neurodevelopment in adolescence (Arseneault, Cannon, Witton, & Murray, 2004). Epidemiology provides important data generating candidate environmental exposures but is insufficient at proving them and will never be able to address the biological complexity present in schizophrenia (McGrath & Susser, 2009). With the knowledge that schizophrenia is characterised by a wide range of variable risk factors researchers can only begin to elucidate the causal mechanisms of each risk factor when approached from a cross-disciplinary approach (i.e. neurobiological and epidemiological frameworks) to explore how these identified environmental risk factors contribute to the development of schizophrenia.

Since environmental “hits” are only a small piece of the pathophysiological puzzle contributing to the development of schizophrenia, other risk factors must be at play. Genetic influences, which account for 80% of the liability to develop
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schizophrenia, are believed to have a causal influence in the anatomical disturbances observed in schizophrenia (Cannon, 2008a). Studies of monozygotic twins discordant for schizophrenia have revealed promise in disentangling genetic and environmental variation (Plomin & Kosslyn, 2001) and elucidating the neuropathology of a predisposing genotype (Cannon, et al., 2002). Other genetic-risk studies examine the genetic liability of the disease through studying unaffected first-degree relatives (i.e. offspring and siblings) of the proband (i.e. schizophrenia patient). There has been substantial increase in research over the last decade into the familial risk factors which influence the development of psychosis. These studies provide useful putative endophenotypes related to the susceptibility of the illness. However, a genetic predisposition alone cannot predict the transition to psychosis. Representative samples of first-episode patients have found that only 10% had a positive family history of the disease (Cannon & Keller, 2006). One report revealed that two-thirds (63%) of individuals diagnosed with schizophrenia do not have a first or second degree relative with the illness (Gottesman & Erlenmeyer-Kimling, 2001). Furthermore, this methodology of studying adult relatives of schizophrenia probands, who are individuals that have already passed the normal age threshold for risk, does not allow researchers to study individuals who are at-risk and observe early risk markers but are instead measuring behavioural traits of who do not develop schizophrenia. Therefore, although genetic high-risk samples help to identify core biological markers of the illness, genetic transmission is not a strong enough vulnerability marker alone. Future studies need to assess environmental and clinical risk factors in individuals who have not yet passed through the normal age window for risk to allow for neurodevelopmental differences to be expressed and longitudinally observed.
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Structural neuroimaging research exploring the progressive changes in the brain leading to the development of schizophrenia support the hypothesis that early neurodevelopmental structural lesions interact with the new demands placed on the brain during pubertal development (e.g. prefrontal cortex recruitment during working memory) cause further behavioural impairments (Pantelis, Yücel, Wood, McGorry, & Velakoulis, 2003). During late neurodevelopment individuals experiencing functional impairments begin to decline more rapidly as the changing requirements placed on their brain are not supported by the same plasticity that those who did not incur an early neurodevelopmental lesion. Later in this chapter I describe specific structural and functional MRI findings in the risk stage that may serve as potential neurodevelopmental risk markers for the disease which are present before and after onset.

1.3.2 Dopamine hypothesis

A widely accepted theory regarding the molecular pathology of psychosis is the dopamine theory of schizophrenia first discovered by Carlsson and Lindqvist (1963) when they studied the properties of antipsychotic drugs. They found that these drugs increased the metabolism of DA. Additionally, evidence comes partly from the discovery of a class of drugs called the phenothiazines, which block DA receptors ($D_2$), which can reduce psychotic symptoms. Also, studies of illicit drugs, such as amphetamine and cocaine, which are known to greatly increase DA levels, can evoke psychotic symptoms (Lieberman, Sheitman, & Kinon, 1997). Due to these properties, most modern antipsychotic medications, for example, risperadal, are designed to block DA function to varying degrees.

A dysfunction of dopamanergic innervation in the prefrontal cortex (PFC) has been linked to aberrant developmental processes (Weinberger, 1987). However,
hypodopamanergia stands in contrast to the therapeutic properties of antipsychotics which block D\textsubscript{2} receptors. Resolving this possible contrast, studies of subcortical DA function have revealed hyperdopamanergia in the presence of a depletion in PFC DA (Carter& Pycock, 1980), suggesting that the antipsychotics that block DA receptors counteract the increased dopamanergic tone in subcortical regions. Davis et al. (1991) parsed out theorises of the dopamine hypothesis, which have revealed hypo- and hyperdopaminergia by theorising that the negative symptoms in schizophrenia result from reductions in DA levels in the PFC, whereas positive symptoms result from the striatal increases in DA.

The mesolimbic DA pathway, which includes the hippocampus, amygdala, nucleus accumbens (Nacc), and ventral tegmental area (VTA) has been associated with schizophrenia due to its role in emotional processing, learning, and reward salience attribution (Laviolette, 2007). Reward prediction has been linked to the role of midbrain DA firing in reward anticipation (Pierce & Kumaresan, 2006; Schultz, 2002). Increases in DA have been related to motivational salience by linking reward stimuli over a longer time scale, giving rise to associations between environmental stimuli and "stamping in" these associations (Berridge & Robinson, 1998). Therefore reward associated stimuli attract the attention of an individual and make that stimulus salient to the individual, which in turn drives their goal-directed behaviours. Salience attribution has been shown to be aberrant in schizophrenia patients (Kapur, Mizrahi, & Li, 2005). Kapur (2003) theorised that during the prodromal stage of psychosis the context-driven activity of the DA system is dysfunctional, which can arise from an environmental and/or genetic insult, which leads to context-inappropriate firing and release of DA producing a perplexing sense of novelty in the prodrome. Abnormally high DA levels in midbrain regions have been strongly linked not only to those
diagnosed with schizophrenia, but in individuals in the prodromal phase of the illness (McGuire, Howes, Stone, & Fusar-Poli, 2008) and in genetically high-risk individuals, who show an increase in pre-synaptic DA in the dorsal striatum (Howes, Montgomery, Asselin, Murray, Grasby, & McGuire, 2007). Laruelle (2000) reported that among individuals experiencing an acute first-episode they show increased DA in the striatum at the site of the synapse. Localisation of pre-synaptic DA was examined in a positron emission tomography (PET) study of at-risk mental state (ARMS) and first-episode patients (Mean age: 26 years old) which found that both groups had elevated DA levels in the dorsal striatum (an association region), but not in the ventral striatum (limbic region) or posterior striatum (sensory motor regulation), relative to a control group (Howes, et al., 2009). This localisation in a sub-region of the striatum implicates the specificity of DA transmission in individuals with psychosis, since all three of these striatal regions receive DA inputs. Cytoarchitecturally, the dorsal striatum receives input from the substantia nigra, whereas the ventral striatum receives its DA input from the VTA. O’Doherty and colleagues (2004) have attempted to disentangle the roles of reward learning in the ventral and dorsal striatum using fMRI. They concluded that the former serves as a “critic” that has the primary role of reward prediction and anticipation whereas the latter is the “actor” who is involved in the modulation of the stimulus-response reward association. The integrative role of the dorsal striatum has been suggested to be the funnelling of motivational information from the "limbic" system to the motor system (Mogenson, Ciriello, Garland, & Wu, 1987), thereby mediating the effects of stimulus-reward mechanisms on goal-directed behaviour (Robbins, Cador, Taylor, & Everitt, 1989; Schultz, 1992). A combined study of fMRI and PET in individuals at-risk found that during a task of verbal fluency the ARMS group had an increase in left inferior frontal
gyrus (IFG) functioning during word production which was correlated with an increase found in DA levels in the dorsal striatum relative to controls (Fusar-Poli, 2009b). Recently, Howes and Kapur (2009) have reviewed the literature of the role of DA in schizophrenia and proposed a revised dopamine hypothesis in light of new evidence. Figure 1.2 summarises their hypothesis by highlighting four pillars of their theory. Firstly, stating the role of the neurodevelopmental hypothesis in identifying multiple “hits” which interact and result in DA dysregulation. Secondly, they have found that DA dysregulation is no longer primarily found at the D2 receptor level, but instead found in pre-synaptic dopaminergic control level, particularly in the dorsal striatum sub-region. Thirdly, DA dysregulation is not specific to schizophrenia, but rather psychotic-illnesses and psychotic-proness. Lastly, DA dysregulation gives rise to altered appraisal of stimuli, which is possibly due to aberrance in salience attribution. They conclude in their hypothesis that changes in multiple neural pathways, between the frontal and striatal regions, converge with biological and environmental influences which lead to the hyperfunction of striatal DA to give rise to psychosis and the subsequent features that constitute a diagnosis of schizophrenia.

Figure 1.2. Dopamine hypothesis of schizophrenia. Multiple hits interact to result in striatal dopamine dysregulation to alter the appraisal of stimuli and resulting psychosis, whilst current antipsychotic drugs act downstream of the primary dopaminergic dysregulation. Reprinted from Howes and Kapur (2009).
Work by Paterlini et al. (2005) sought to find DA and glutamate systems contribute to the development of schizophrenia. They found that the PRODH gene on region q11 of chromosome 22 is deficient, likely due to a micro-deletion on the chromosome. This creates high proline levels, which mimic glutamate to produce excitatory activity. When this occurs the normal compensatory upregulation of the cathechol-o-methyltransferase (COMT) gene, also located on chromosome 22 and which encodes for the enzyme that breaks down DA, cannot occur resulting in an abundance of DA in the brain. The result of this PRODH deficiency was highlighted when increased glutamate was found at synapses formed by CA3 and CA1 neurons in the hippocampus. The hippocampus is involved in regulating activations in the brain responsible for learning and memory. The increased release of excitatory chemicals glutamate and proline due to PRODH deficiency inhibited the ability of the synapse to undergo a change called long-term potentiation. This phenomenon creates the long-lasting strength of connection between two neurons. Long-term potentiation is an important step in forming memories, and disruption of this process interferes with the ability to store information. Moreover, the researchers showed that PRODH deficiency caused a reduction in the levels of three proteins that, in combination, are associated with DA function in the frontal cortex. This model provides evidence of a probable cause of DA dysregulation in schizophrenia, in which a deficiency in PRODH and COMT, due to a micro-deletion on chromosome 22q11 interact to control DA levels.

1.3.3 Disconnection hypothesis

Schizophrenia is characterised by widespread deficits in grey and white matter associated with distributed functional abnormalities (see meta-analysis in Wright, et al., 2000). Such observations have prompted the idea that schizophrenia is a
disconnection syndrome adversely affecting the structure of neurocognitive networks in the brain (Bullmore, Frangou, & Murray, 1997; Friston, 1998; Friston & Frith, 1995). The disconnection hypothesis has received copious amounts of empirical support from functional brain imaging studies and points to potential mechanisms at the synaptic or molecular level however there is a lack of consensus on which brain networks are the site of the primary abnormality. Some investigators propose that the core abnormality is a disruption of frontal-temporal integration (Friston & Frith, 1995) while others propose a disruption of cortico-cerebellar-thalamo-cortical circuitry (CCTCC) integration (Andreasen, et al., 1999). Another model suggests that fronto-temporo-limbic interactions with the ventral striatum are impaired (Buchsbaum, 1990; Csernansky & Bardgett, 1998), which could partially be explained by the dopamine hypothesis. The parietal lobes have been less emphasised in schizophrenia research but there is emerging evidence that fronto-parietal integration may be abnormal (Hugdahl, et al., 2004; Quintana, et al., 2003). Yoon et al. (2008) found a pattern of connectivity in controls, between dorsal lateral prefrontal cortex (DLPFC) and right inferior parietal lobule during context processing when performing a continuous performance test (CPT), the AX-CPT, however schizophrenia patients did not show any regions with enhanced functional connectivity to the DLPFC.

Recently, Benetti et al. (2009) explored effective connectivity during a working memory task in first-episode, at-risk and healthy control subjects. They set out to assess bi-directional connectivity between the PFC and hippocampus, particularly between the IFG which has prominent projects to the posterior subdivision of the hippocampus. They examined the phase of correct recognitions to previously presented stimuli and found reduced effective connectivity from the right posterior hippocampus to the right IFG in the first-episode and at-risk subjects,
compared to controls. Findings of decreased intrinsic connectivity in subjects at high-risk is consistent with reports of abnormal PFC, posterior, and also subcortical brain region functional connectivity (Whalley, et al., 2005). Other recent reports have associated the ARMS with focal reductions in PFC and medial temporal cortices (Borgwardt, et al., 2007; Broome, et al., 2009; Pantelis, Yücel, Wood, McGorry, & Velakoulis, 2003). Disruptions in fronto-hippocampal connectivity are rapidly becoming a compelling vulnerability marker in individuals who are at-risk. Studies which explore the cortical and subcortical dysfunctions stemming from the frontal cortex with connections to temporal, parietal, limbic and striatal regions will be explored in greater detail in this chapter.

1.4 Vulnerability for psychosis

Beginning in the mid-20th century a shift in psychosis research occurred and investigators began to focus on the aetiology of psychosis in individuals who did not have the established illness, but rather possessed hypothesised risk factors that could lead to the development of psychosis. New primary data regarding the phases of clinical transition to psychosis have prompted the review of some long-held views about the epidemiology of schizophrenia. Recently, a new approach to investigating potential causal mechanisms leading to psychosis has taken a different direction by studying individuals who report subclinical psychotic symptoms. These attenuated psychotic symptoms may be the first identifiable antecedents to the full-blown expression of the illness.

Retrospectively, the time which precedes the first-episode is known as the “prodromal” phase. Anytime before the first-onset of the disease is also known as the “premorbid” phase. Symptoms reported in this phase include attenuated psychotic symptoms, increased depressive symptoms, a decline in general functioning, and
cognitive disturbances (McGlashan, 1996). Prospectively, the prodromal phase has been identified as the “ultra-high risk” (UHR) phase, based on meeting specific criteria which are predictive of the transition to psychosis. Individuals who are identified in the UHR phase are referred to as being in the at-risk mental state (ARMS) if they meet specific diagnostic criteria (Table 1.2). However, meeting ARMS criteria does not guarantee that the individual will go on to develop psychosis with transition rates to psychosis estimated to be between 10-35% over a period of 12 months to three years follow-up (Cannon et al., 2008b; McGlashan, Miller, & Woods, 2001; Velthorst, et al., 2009; Yung, et al., 2003; Yung, Phillips, Yuen, & McGorry, 2004).

1.4.1 Ultra-high risk/prodromal phase

Perceptual hypersensitivity and cognitive deficits are believed to start before the onset of frank psychosis (Carpenter & Kirkpatrick, 1988; Yung & McGorry, 1996). Nearly 75% of the total schizophrenia cases begin with a prodromal phase, lasting an average of five years, with their psychotic-like symptoms lasting approximately 1.1 years before onset of this disease (Hafner, 1998). Investigators have found that patients typically experience active psychosis for 12-24 months before admission to the hospital or other acute treatment is obtained (Fenton, Blyler, & Heinssen, 1997; Gift, Strauss, Harder, Kokes, & Ritzler, 1981). Since the onset of schizophrenia is believed to occur during late adolescence to early adulthood the prodromal stage therefore begins at an age in which social and cognitive development and brain maturation are still ongoing. The negative prodromal symptoms are often associated with social and cognitive deficits already at this stage.
Table 1.2

UHR status

Group 1: Attenuated psychosis group

(i) Subthreshold intensity:
- Severity scale score of 3–5 on disorders of thought content subscale, 3–4 on perceptual abnormalities subscale and/or 4–5 on disorganized speech subscale of the CAARMS;
- Frequency scale score of 3–6 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale of the CAARMS for at least 1 week; OR
- Frequency scale score of 2 on disorders of thought content, perceptual abnormalities and disorganized speech subscale of the CAARMS on more than two occasions.

(ii) Subthreshold frequency:
- Severity scale score of 6 on disorders of thought content subscale, 5–6 on perceptual abnormalities subscale and/or 6 on disorganized speech subscale of the CAARMS;
- Frequency scale score of 3 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale of the CAARMS; (for both categories)
- Symptoms present in past year and for not longer than 5 years.

Group 2: BLIPS group:

- Severity scale score of 6 on disorders of thought content subscale, 5 or 6 on perceptual abnormalities subscale and/or 6 on disorganized speech subscale of the CAARMS;
- Frequency scale score of 4–6 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale;
- Each episode of symptoms is present for less than 1 week and symptoms spontaneously remit on every occasion;
- Symptoms occurred during last year and for not longer than 5 years.

Group 3: Vulnerability:

- Family history of psychosis in first degree relative OR schizotypal personality disorder in identified patient;
- 30% drop in GAF score from premorbid level, sustained for 1 month;
- Change in functioning occurred within last year and maintained at least 1 month.

Psychotic disorder threshold:

- Severity scale score of 6 on disorders of thought content subscale, 5 or 6 on perceptual abnormalities subscale and/or 6 on disorganized speech subscale of the CAARMS;
- Frequency scale score of greater than or equal to 4 on disorders of thought content, perceptual abnormalities and/or disorganized speech subscale;
- Psychotic symptoms present for longer than 1 week.

The prodromal stage has gained increasing interest from an intervention standpoint. It has long been known that the reduction in time of the duration of untreated psychosis improves clinical outcome (Sheitman, Lee, Strous, Strauss, & Lieberman, 1997). Studies of this phenomenon have revealed that the longer the duration of untreated psychosis predicted poorer long-term outcome in regards to distractibility (Harrison, Croudace, Mason, Glazebrook, & Medley, 1996), increased severity of negative symptoms, such as poverty of speech (Waddington, Youssef, & Kinsella, 1995), and morbidity of the disease (McGlashan & Johannessen, 1996; Wyatt, 1995). Recently, studies are investigating the efficacy of intervening before the onset of the first-episode. Morrison and colleagues (2004) identified a sample of...
individuals in the ARMS and administered cognitive-behavioural therapy (CBT) to one-half of the participants or no treatment and just monitoring of the other half. At a 12-month follow-up 26% of those not administered CBT met DSM-IV diagnostic criteria for psychosis within the study period whereas only 6% of those treated with CBT converted to psychosis. Studies of intervention during the prodromal phase gives validation to the identification of individuals in this vulnerable stage and the impact this may have on hindering the progression to the disease.

1.4.2 Attenuated psychotic symptoms

Recent research has suggested that although strict ARMS criteria provide good predictive value, such intensive clinical batteries may not be necessary to detect the beginning phase of psychosis. Attenuated, or transient, psychotic symptoms, also referred to as subthreshold, subclinical basic symptoms, or psychotic-like symptoms, which do not meet the frequency and duration criteria for a clinically defined psychotic illness, have begun to show promise as isolated predictors of risk. However, some controversy surrounds this method of identification as the incidence of psychotic-like symptoms in the general population is quite high, as will be discussed in the following paragraph. Although these psychotic symptoms do not meet threshold criteria for clinical significance, they may be a signpost for the detection of the earliest vulnerability markers of the disease revealing a unique stage of risk which is free of chronicity and medication confounds. Research has revealed that the longer the duration of untreated psychotic symptoms the poorer long-term outcome is for the patients who convert to psychosis (Birchwood, Todd, & Jackson, 1998; Perkins, Gu, Boteva, & Lieberman, 2005), therefore early identification is paramount.

Research into the incidence of psychotic experiences has revealed that a continuum exists between psychotic and ordinary experiences (Chapman & Chapman,
Epidemiological studies have revealed a wide range, between 4-71%, of the general population report hallucinations or delusions with a range of severity and frequency over their lifetime (Barrett, 1992; Johns, Nazroo, Bebbington, & Kuipers, 1998; McKellar, 1968; Posey & Losch, 1983; Poulton, et al., 2000; Tien, 1991; van Os, Hanssen, Bijl, & Ravelli, 2000; van Os, Linscott, Myin-Germeys, Delespaul, & Krabbendam, 2009). However, the estimated lifetime risk of schizophrenia is under 1% (Jablensky, 1995; McGrath, 2005). Furthermore, approximately 28% of the individuals from the U.S. National Co-morbidity Survey admit experiencing a psychotic-like experience (i.e. hearing voices at least once a month), but when interviewed by a clinician there was only a 0.7% rate of psychosis diagnosed in that subgroup (Kendler, Gallagher, Abelson, & Kessler, 1996). Additionally, another population-based study of individuals, who were aged 18-64, found that 17.5% have experienced a psychotic symptom, of those who experienced psychotic symptoms only 2% were diagnosed with a non-affective psychotic disorder. Yung et al. (2009) recently found that in a population-based study of adolescents in their tenth academic year (aged 13-18) (n= 875) found that 28% of this sample experienced psychotic-like symptoms, however, when questioned on the frequency of these events only 1.9% reported that they regularly experienced these experiences. Additionally, van Os et al. (2001) found that in individuals at-risk for psychosis those with more frequent psychotic-like symptoms have worse behavioural functioning than those with less frequent experiences. However, the pathology of these psychotic experiences cannot be overlooked due to the fact that those same symptoms, although not as severe or debilitating, are the core features experienced by those diagnosed with a psychotic disorder. Research conducted by van Os and colleagues (2000; 2009) has suggested that those with diagnosed psychotic symptoms in clinical samples are part revealing
part of a continuum of experiences, which also appear in the general population. The work of van Os et al. (2009, p. 190) stated that "these studies of [subclinical psychotic symptoms] demonstrate that the generally good (because the symptoms are only transitory as described above) outcome of subclinical psychotic experiences can be modified to poorer outcomes of persistence and clinical need for care if subjects are exposed to additional (proxy) environmental risk factors." Figure 1.3 represents the hypothesis of van Os et al. (2009) the phenomenon of persistence and subsequent development of a psychotic illness and the need for clinical intervention. This diagram depicts a diagnosable psychotic disorder, as related to the processes of biological and psychological sensitisation, and explains differences in longitudinal trajectories of psychosis proneness.

![Figure 1.3. Sensitisation and onset of psychotic disorder. Person A has 'normal' developmental expression of subclinical psychotic experiences (psychosis proneness) that are transient. Person B has similar expression but longer persistence due to additional but mild environmental exposure. Person C has longer persistence due to severe repeated environmental exposure and transition to clinical psychotic disorder with significant impairment. Reprinted from van Os et al. (2009).]
The description of psychotic-like experiences can be best illustrated by actual reports from individuals experiencing psychotic-like events. In the following chapters report the work from a population-based study of school-age children (aged 11-13) in the Adolescent Brain Development (ABD) Study who were screened for the incidence of psychotic experiences. During a semi-structured clinical interview, the Schedule for Affective Disorders and Schizophrenia for School-Aged Children (K-SADS) (Kaufman, et al., 1997), the children who were identified as experiencing a “definite” psychotic symptom revealed the reality of their troubling psychotic experiences. Kelleher et al. (2009) described the findings from the clinical interviews of some of the children found to be at-risk. He described that one girl heard the sound of her own angry thoughts coming from outside her head twice a year for two years. One boy experienced hearing the voices of friends from a school he previously attended yelling unintelligible things all at once; he would suspect that someone was following him. Another child heard odd-sounding girls’ voices and believed that others were talking about her and this occurred approximately once every six months; she felt chills and believed that a ghost was passing through her. These psychotic-like experiences are similar to those experienced by schizophrenia patients, however, the frequency and behavioural impairment of these experiences does not meet criteria for a psychotic disorder.

Research from longitudinal population-based studies which surveyed psychotic-like experiences in the general population have begun to surface. Longitudinal research has shown that children who report subclinical psychotic symptoms are at an increased risk, ranging from 16-to 65-fold increase, to later develop a clinical psychotic disorder (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005; Poulton, et al., 2000). In a general population-based study in Munich, Germany
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(Dominguez, Wichers, Lieb, Wittchen, & van Os, 2009), a sample of 845 adolescents, aged 14–17 years, were assessed for psychotic symptomatology four times over a period of eight years. They found that subclinical psychosis was confirmed to be common, occurring in 22% of the sample but mostly transitory (i.e. only occurred once or twice), with recurrence or persistence occurring in 30–40% of those with symptoms. Additionally, of those diagnosed with psychosis at the final intake (n= 47), 38% had symptoms that were identifiable in the first three intakes, and 20% had symptoms that occurred more frequently. Therefore, clinically relevant psychosis in young adults could be traced to the subclinical psychosis phenotype expressed up to eight years earlier, with the occurrence of the symptoms to be a strong indicator of those whose symptoms will persist.

An Australian birth cohort study (Scott, et al., 2009) followed 7,223 mothers and their offspring for 21 years. Information was derived from the Diagnostic Interview Schedule for Children (DISC-IV) (Costello, et al., 1982) and the Child Behavioural Checklist (CBCL) which collected information on psychopathology which came from mothers’ ratings of their children’s emotional and behavioural problems at ages five and 14 and from the children’s self-reports at age 14 and at age 21. The children also specifically were asked questions about delusions on a screening questionnaire. Hallucinations were reported by 8.4% of adolescents, who were aged 13-17. They found that the frequent experience of visual or auditory hallucinations at age 14 strongly predicted delusion-like experiences at age 21 (OR for auditory hallucinations= 4.84, 95% confidence interval (CI): 2.08-11.26; for visual hallucinations OR = 8.68 (95% CI: 2.57-29.30) (Wilcox, 2009).

In a New Zealand birth cohort (Poulton, et al., 2000) 11-year olds who reported psychotic symptoms were at 16-fold increased risk of adult psychotic illness.
Twenty-five percent of the children who displayed definite symptoms at age 11 were diagnosed with a schizophrenia-spectrum disorder by age 26. A population-based study in the Netherlands (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005) in adults aged 18-64, found that in those who experienced subclinical psychotic symptoms they were at a 65-fold increased risk to develop psychosis, with 8% of those with psychotic-like symptoms transitioning to a clinical psychotic disorder at two-year follow-up. The two-year risk rose to 21% for those with multiple psychotic experiences, and to 15% for those whose psychotic experience had arisen in the context of significant lowering of mood.

In a population-based sample of children (Abdellaoui, Bartels, Hudziak, Rizzu, van Beijsterveldt, & Boomsma, 2008), who were aged 7-8 years old, 9% reported auditory hallucinations, which were predominantly unknown to most of their parents. In regards to impairment in functioning most of the children experienced little distress or disruption from the experience of the hallucinations. However, 15% reported intense suffering and 19% reported that the hallucinations severely impacted their thinking. Interestingly, the children who experienced motor developmental delays had increased odds of hearing voices (OR: 1.22, 95% confidence interval 1.02-1.46). In regards to behavioural associations with the symptoms, children with severe auditory voice hallucinations experienced more somatic complaints than those with either mild or no such hallucinations (OR: 1.25, 95% confidence interval 1.03-1.52).

However, there is some controversy surrounding the identification of this risk group given the high proportion of adults who endorse psychotic-screening questions. In Chapter Two I report the neuroimaging results from a group of school-age children (aged 11-13) who were screened for the incidence of psychotic experiences through the use of an instrument which we called the Adolescent Psychotic-Like Symptom
Screener (APSS) (see Chapter Two, Figure 2.2). Kelleher and colleagues (2009) reported a validation of the sensitivity and specificity of the questionnaire. His analysis included a slightly larger number of participants than reported in Chapter Two (n= 334 vs. n= 277). His study compared responses to the seven-item screening questionnaire with data from semi-structured interviews that were based on the K-SADS. In response to the screening questions, 38% of the children endorsed “yes, definitely” for at least one item from the APSS. From the interviews, 17 subjects reported “definite” psychotic-like experiences and five reported “possible” ones, and these 22 subjects comprised the at-risk group, revealing that about 7% of those screened were identified as at-risk. The single most reliable APSS question in identifying an individual who was at-risk, as confirmed by the follow-up clinical interview, was the question regarding hallucinations (i.e. “Have you ever heard voices or sounds no one else can hear?”). This question was endorsed by 35% of the sample and attained a positive predictive value of 71% and a negative predictive value of 90% in identifying individuals at-risk. Additionally, among those interviewed, two individuals were confirmed to be in the prodromal phase of the illness (0.7%), which is higher than the estimated annual incidence rate in adults (.02%) (McGrath, Saha, Chant, & Welham, 2008) (Note: The research carried out in this thesis investigated children experiencing subclinical psychotic symptoms; therefore, the two children identified as prodromes were not included in any of the analyses reported.) These findings reveal that the prodromal phase of psychosis can be detected during childhood with the use of a symptom’s screening tool. Additionally, the incidence of detecting psychosis among the general school-age population with the APSS is higher than those reporting the illness who sought out treatment (i.e. 0.7% vs .02%). In chapters Three, Four and Five I describe multi-modal neuroimaging approaches to
elucidating the neuropathology of these individuals presenting psychotic-like symptoms, which may in turn, reveal neurobiological vulnerability markers of schizophrenia.

1.5 Magnetic resonance imaging (MRI)

MRI is the leading research tool guiding research into the vulnerability for allows psychosis today, creating an abundance of related literature into the risk for psychosis. It for the investigation of baseline measures of volume, function, structure, and connectivity in the tissues of the brain. The soft tissue contrast in MRI is believed to be superior to the previously dominant imaging technique of computed tomography (CT), which utilised ionizing radiation to construct images. MRI images are created through the use of pulse sequences, which are created through changing the magnetic field gradients and fluctuating electromagnetic fields. The frequency of the pulse sequence affects the atomic hydrogen nuclei in the tissue, which are the most common nuclei in humans as they are a main component of the water molecule. The water molecules have a magnetic property which causes spin dephasing, when introduced into a magnetic field (Huettel, Song, & McCarthy, 2004). When outside of the scanner the water molecules of the brain are spinning in random directions on their axes (Figure 1.4A). However, when the atomic nuclei absorb the electromagnetic activity produced by the scanner they behave like magnetic spinning tops and are forced to align in the same direction (Figure 1.4B) but can be disturbed from this orientation by a burst of energy from a radio frequency transmitter, located in the MRI transmitter coil.

The biological underpinning of fMRI relates to the metabolic demands of active neurons. The technique, which forms the basis of fMRI, is known as the blood oxygen-level dependant (BOLD) contrast. This contrast was discovered by Ogawa
and Lee (1990), who were the first to hypothesise that changes to the proportion of blood oxygen could enhance the appearance of blood vessels during MRI. Their work was based on the work of Linus Pauling and Charles Coryell who were the first to discover that the hemoglobin molecule has magnetic properties that are attenuated by the presence or absence of oxygen binding to it (referenced in Huettel, Song, & McCarthy, 2004). They found deoxygenated hemoglobin is paramagnetic, meaning the molecules in the blood, which are not attached to oxygen, can be influenced by the intensity of magnetisation when placed in a magnetic field which is sensitive to the time constant (T2*). Ogawa and colleagues (1990) seminal study with rodents either breathing oxygen or carbon monoxide revealed that deoxygenated blood decreased the measure of MR signal on T2* images creating local field distortions which were depicted as thick dark lines in echo images. The complexity of the physiological and
physical response to the fMRI T2* signal is further elaborated upon and represented in Figure 1.5.

Figure 1.5. Schematic of interactions in the formation of the BOLD signal. Positive/negative arrows indicate positive/negative correlations between the parameters. The right most pathway (in bold arrows) is the most significant effect in most BOLD fMRI. Reprinted from Springer et al. (1999).

1.5.1 Structural magnetic resonance imaging (sMRI) in schizophrenia

Shenton et al. (2001) reviewed structural MRI studies in schizophrenia patients from 1990 to the time of publication and identified the most replicated structural alterations associated with the disease. In this review they found the most common abnormality was within the superior temporal gyrus (STG) (100% in GM
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studies); followed by cavum septi pellucid (92%); lateral ventricles (80%); amygdala/hippocampi complex (74%); third ventricles (73%); basal ganglia (68%); corpus collosum (63%); temporal lobe (61%); planum temporal (60%); frontal lobe (60%); parietal lobe (60%); occipital lobe (44%); thalamus (42%); cerebellum (31%); and whole brain volume (22%). The seminal computer topography (CT) brain imaging study conducted by Johnstone and colleagues (1976) was the first to discover enlarged lateral ventricles in schizophrenia patients which were linked to cognitive deficits. Although enlarged lateral ventricles are one of the most replicated structural findings in schizophrenia and appear to be present at onset of the disease (Shenton, Dickey, Frumin, & McCarley, 2001), there is no definitive evidence that this abnormality can be detected in the prodromal phase (DeLisi, 2008). A meta-analysis of 66 first-episode studies (52 cross-sectional and 16 longitudinal studies) (Steen, Mull, McClure, Hamer, & Lieberman, 2006) found volumetric changes in the illness were most commonly found in whole-brain volume and hippocampal volume.

The relationship between structure and function is increasingly becoming a standard reporting measure in studies of cognitive deficits in schizophrenia. A review conducted by Antonova and colleagues (2004), found that between 1990 and the time of publication 35 MRI studies were published which examined the relationship between structure and neurocognition in first-episode and chronic schizophrenia. Their research did not reveal a correlation between absolute ventricular size and cognitive deficits in schizophrenia patients, revealing that the relationship between ventricular size and cognitive functioning may not in itself be causal, but abnormalities in more specific surrounding regions, such as in the thalamus, may give rise to the cognitive abnormalities. It has also been suggested that the ventricular enlargement is secondary to the progression of cortical changes that appear first
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Introduction to the risk for psychosis (DeLisi, 2008). The most common reports are of reductions in grey matter (GM) volume in the PFC in relation to deficits observed in working memory which may contribute to the cognitive deficits of executive functioning, conceptual thinking, and memory formation (Goldman-Rakic, 1995; Goldman-Rakic, 1999; Goldman-Rakic & Selemon, 1997). Antonova and colleagues (2005) found that a specific decrease in GM in the right IFG was associated with decreased verbal memory performance in schizophrenia patients. Antonova and colleagues (2004) found that studies of PFC volume most consistently correlated with tasks of executive functioning, attention, and visual memory in schizophrenia. However, their review neither highlighted that all cognitive disturbances are associated with frontal functionality nor were they always associated with reduced PFC GM volume.

Although structural (and later discussed functional) disturbances of the PFC are frequently reported and emphasised, this brain region is only one of an abundance of other abnormal brain regions found in schizophrenia patients and those at-risk. A study of executive functioning during the Wisconsin-Card Sorting Task (WCST) in schizophrenia patients found that reduced DLPFC and anterior cingulate cortex (ACC) GM volumes were correlated with poorer executive functioning performance (Rüsch, et al., 2007). Additionally, they found that the DLPFC volume in schizophrenia patients was positively related to ACC and parietal GM volume, and negatively correlated with parahippocampus, thalamus, pons and cerebellum GM volume. This study emphasises the PFC as playing part of a larger neural network that does not operate in isolation. This was highlighted through the relationship of the volume of GM in the PFC and other cortical and subcortical regions in the brain. Graybiel (1997) has suggested that the PFC does not have to be structurally altered for functional disturbances to appear. He instead proposed a model which emphasized the
connectivity between the basal ganglia and parallel neural circuits that connect efferent projections from the PFC to the subcortical structures such as the caudate. Antonova et al. (2004) have suggested that a dysfunction in the basal ganglia, whose role has been implicated in the generation of the cognitive blueprints for actions that involve thought, movement and emotion, would lead to disturbances in schizophrenia, which are respectively presented as cognitive, negative and affective symptoms. One explanation posited by Pearlson et al. (1996) suggested that schizophrenia involves a disruption in communication between the PFC and limbic system, thalamus and basal ganglia which manifests in disturbances of higher-order cognitive functioning. Another model which was proposed by Andreasen and colleagues (1996; 1998; 1999) suggested that that the disorganised symptoms of schizophrenia, known as “cognitive dysmetria,” arise from disruptions within the CCTCC in the brain, which is a network that subserves the coordination of both motor and cognitive processes (Middleton & Strick, 2000; Schmahmann, 1991). Andreasen et al. (1999) found evidence that subtle motor dysfunctions, which are evident in the illness and are also premorbid markers of the illness, may reflect a fundamental disruption in the synchrony of thought and action. A functional connectivity study, which used a CPT assessment of attentional modulation in the motor system, explored patterns of activation in schizophrenia patients. They found that patients revealed reduced CCTCC connectivity, in the medial superior frontal gyrus, ACC and cerebellum, compared to healthy controls (Honey, et al., 2005).

Thompson and colleagues (2001) conducted an influential longitudinal sMRI study of childhood-onset schizophrenia that examined the progression of cortical neurodegeneration in the earliest stages of schizophrenia. They found that cortical GM losses began in the parietal lobe, with a significant decrease in volume compared
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to healthy developing adolescents. The GM loss spread through the motor cortices
than dynamically spreading to the frontal lobes engulfing regions along its path,
mainly in the lateral temporal lobes and superior frontal gyrus, over the course of five
years (Figure 1.6). Interestingly, a sub-analysis of GM differences at the beginning of
the study found that the marked progressive DLPFC and lateral temporal deficits were
not apparent at age 13, even though the illness had been present for a mean of three
years (Figure 1.7). This finding suggests that the process of PFC and temporal GM
erosion emerges later in the course of development. However, the initial parietal
deficit, which was also progressive and accelerated in schizophrenia patients,
occurred in similar regions where normally developing adolescents also lose GM.
This study not only identified specific cortical regions with accelerated GM loss, but
also expanded on the normal path of cortical GM found during neurodevelopment in
adolescents (Giedd, et al., 1999). Healthy adolescents lose temporal lobe GM at a rate
of 1-2% per year whereas adolescents with schizophrenia show rapid loss of matter at
a rate of 3-4 % a year (Thompson, et al., 2001; Toga, Thompson, & Sowell, 2006).
This may be due to an increase in neuronal death caused by apoptosis whereby both
neurodegenerative and neurodevelopmental processes are implicated. Temporal lobe
abnormalities have been consistently associated with positive symptoms in chronic
schizophrenia (Shenton, et al., 1992; Turetsky, et al., 1995). A study of adolescent-
onset schizophrenia patients (Douaud, et al., 2007) found a GM reduction in inferior
temporal gyrus (BA20) relative to adolescent controls. Additionally, a study
examining GM volume within first-episode schizophrenic and affective psychosis
patients revealed both groups had a reduction in inferior temporal lobe GM volumes,
therefore revealing an underlying structural commonality within individuals with
psychotic symptoms (Kuroki, et al., 2006b). Conversely, a study of children with
Figure 1.6. Average rates of gray matter loss in normal adolescents and in schizophrenia. (A) Three-dimensional maps of brain changes, derived from high-resolution magnetic resonance images (MRI scans) acquired repeatedly from the same subjects, reveal profound, progressive gray matter loss in schizophrenia (Right). (B) Average gray matter loss rates were computed for 24 subjects in superior frontal gyri (SFG), lateral temporal cortices (LTC), and superior parietal lobules (SPL) in both brain hemispheres. Error bars indicate the standard error of the sample means, by region, in controls and patients. Individual loss rates (in percent per year) are plotted (■, patients; □, controls), showing significant group separation, despite some outliers. Reprinted from Thompson et al. (2001).
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Early and late gray matter deficits in schizophrenia

Deficits occurring during the development of schizophrenia are detected by comparing average profiles of gray matter between patients and controls at their first scan (age 13; A) and their last scan five years later (age 18; B). Although severe parietal, motor, and diffuse frontal loss has already occurred (A) and subsequently continues, the temporal and DLPFC loss characteristic of adult schizophrenia is not found until later in adolescence (B), where a process of fast attrition occurs over the next five years. The colour code shows the significance of these effects. Reprinted from Thompson et al. (2001).

Figure 1.7. Mapping early and late grey matter deficits in schizophrenia. Deficits occurring during the development of schizophrenia are detected by comparing average profiles of gray matter between patients and controls at their first scan (age 13; A) and their last scan five years later (age 18; B). Although severe parietal, motor, and diffuse frontal loss has already occurred (A) and subsequently continues, the temporal and DLPFC loss characteristic of adult schizophrenia is not found until later in adolescence (B), where a process of fast attrition occurs over the next five years. The colour code shows the significance of these effects. Reprinted from Thompson et al. (2001).

childhood-onset schizophrenia (Jacobsen, et al., 1996) reported a similar effect of increased temporal lobe volumes in patients, specifically in the superior temporal gyrus (STG). Douaud et al. (2009) explored longitudinal changes in GM over a period of two and a half years in adolescent-onset schizophrenia patients. They found that the schizophrenia patients failed to reveal the same neurodevelopmental time course as healthy adolescents. Instead of the expected reduced GM findings, they found increased GM in sensorimotor regions in patients relative to controls. Greenstein et al. (2006) also found similar increases in GM in a larger sample (n=70) of early-onset schizophrenia patients. These findings are contradictory to Thompson et al. (2001)
1.5.2 sMRI in individuals at-risk

The Edinburgh High Risk Study (Job, Whalley, Johnstone, & Lawrie, 2005) which identified individuals at genetic-risk for developing psychosis found that those who subsequently went on to develop schizophrenia had reduced GM in the inferior temporal gyrus. However, the most common GM reductions in studies of ARMS individuals who later convert to psychosis have found reductions in the ACC (Borgwardt, et al., 2007, Fornito, et al., 2008; Pantelis, et al., 2005). Borgwardt et al. (2007) found reductions in GM in the posterior cingulate, insula, inferior frontal gyrus, and increases in the posterior parietal/temporal lobe in ARMS participants who later converted to psychosis. Takahashi et al. (2009) conducted a longitudinal comparison of the insular cortex GM changes in 31 UHR individuals (20 who subsequently developed psychosis and 11 who did not convert) and 20 controls for whom follow-up MRI data between one and four years later. They found the at-risk subjects who developed psychosis had a significantly reduced right insular GM volume compared with those at-risk who did not convert and healthy controls. More severe negative symptoms in the participants who converted to psychosis were correlated with smaller volumes of the right long insular cortex. These results suggest that GM volume deficits in individuals at risk and around the time of transition from prodromal state to psychosis have structural abnormalities in the inferior frontal gyrus, temporal lobe, cingulate and insula which are present, at least in part, before the onset of the illness and are likely to be linked to disruption of normal brain developmental processes. Parietal deficits have been less well studied in schizophrenia which tends to emphasise PFC and higher-order cognitive abnormalities. However, there is evidence
that volumetric reductions in the parietal lobe are also evident in adult schizophrenia patients but not in their genetically identical non-affected twins, indicating that environmental factors may be involved in the vulnerability to the parietal abnormalities of the disease in adults (Cannon, et al., 2002).

Investigations of GM in monozygotic twins discordant for schizophrenia have also revealed reductions in focal GM reductions in the hippocampus that are specific to trait marker for the disease (Narr, et al., 2002). Disease-specific GM reductions in monozygotic twins discordant for schizophrenia were identified in areas of the DLPFC, responsible for eye movements and motor planning, and in temporal and parietal areas that support multi-modal sensory and perceptual integration and episodic memory (Cannon, et al., 2002). Investigations relating structural and cortical activity in ARMS individuals have revealed that reduced GM in the orbitofrontal gyrus correlated with reductions in working memory abilities (Pflueger, Gschwandtner, Stieglitz, & Riecher-Rössler, 2007). However, these studies have been conducted in post-pubescent individuals, which may explain why PFC deficits in GM were not commonly observed in the at-risk studies reported above, some of which were conducted in younger prepubescent samples of ARMS individuals.

1.5.3 fMRI and cognitive functioning in schizophrenia

Cognitive functioning in schizophrenia has long been characterised by broad neuropsychological impairments with varying degrees of deficit in all ability domains tested, supporting the notion of the disconnection hypothesis. However, prior to the advent of fMRI as a research tool, the localisation of such cognitive disturbances was largely unknown. Some researchers have hypothesised that deficits in cognitive processing arise from a fundamental disturbance in working memory abilities, which are compromised due to abnormalities of DLPFC functioning (Glahn, et al., 2005;
Meyer-Lindenberg, et al., 2001). Neuroimaging has also elucidated that deficits in selective attention, context processing, and response inhibition in schizophrenia patients could arise from PFC functional abnormalities (Alain, Hargrave, & Woods, 1998; Carter, MacDonald, Ross, & Stenger, 2001; Ford, et al., 2004; Potts, O'Donnell, Hirayasu, & McCarley, 2002) which are also found in neuroleptic naïve patients, specifically within the IFG in the ventral lateral prefrontal cortex (VLPFC) (Ojeda, et al., 2002). Figure 1.8 depicts the regions of the PFC, including the DLPFC, VLPFC, and ACC.

Figure 1.8. Cytoarchitectonic maps rendered on the lateral surface and on the medial wall of the PFC. Numbers refer to Brodmann areas (BA). (A) Within lateral surface the DLPFC comprises BA 9/46, BA 46, and BA8a in the middle frontal gyrus), and VLPFC comprises BA 44, BA 45, corresponding to the pars opercularis and pars triangularis of the inferior frontal gyrus, respectively), BA 47/12. (B) On the medial wall, the ACC comprises BA 32, BA 24, and medial frontal gyrus which comprises BA 6, which is divided into the supplementary motor area (SMA) the the pre-SMA and the frontal eye fields (medial BA 8), and dorsomedial PFC (BA 9). Reprinted from Ridderinkhof et al. (2004).

Functional neuroimaging research over the last two decades to elucidate the complex neural basis of schizophrenia has provided an emerging picture of the underlying neuropathology of the illness. The most consistent neuroimaging finding in schizophrenia has been functional abnormalities within the PFC (see review in Hill,
et al., 2004), especially within the DLPFC and VLPFC during tasks of executive functioning (Minzenberg, Laird, Thelen, Carter, & Glahn 2009). However, conflicting findings of hyper- and hypofrontality have been found in schizophrenia patients. Hypofrontality has been attributed to participants performing poorly on a task, in which the demand of the task exceeds the participant’s abilities, and is evidenced by reduced activity within the DLPFC in schizophrenia patients (Minzenberg, Laird, Thelen, Carter, & Glahn 2009). However, hyperfrontality has also been observed in schizophrenia patients and is thought to be a result of inefficient and less automatic processing within the PFC (Weinberger & McClure, 2002). A possible explanation for this phenomenon is the inefficiency hypothesis, which states that when there is impairment in the region that is expected to be engaged for the task (i.e. the right IFG during response inhibition tasks or the DLPFC during context processing), the patients engage more cortical resources in order to maintain a comparable level of performance to controls (Callicott, et al., 2003a). Ramsey and colleagues (2002) have suggested that the inefficiency of neural communication observed in schizophrenia patients results in excessive recruitment of neural systems to perform at comparable levels to controls during cognitive tasks. In a study of working memory functioning during an n-back task in first-episode patients, researchers found that during the 0-back condition first-episode patients activated the same extensive network of activity as the controls during the 2-back condition, but found overall reduced activity in the patients during the 2-back condition (Mendrek, et al., 2004). Thus, the normally undemanding 0-back task required patients to recruit available cerebral resources to near maximal capacity therefore causing relative hypoactivity in the patients these regions exhausted for the demanding 2-back condition. This is due to patients using all cognitive/cortical resources for the easier version of the task and therefore not
leaving any additional resources to recruit as the task becomes more demanding. Therefore, regions that are hypothesised to be hypoactive in patients during the task, such as the posterior regions, are recruited to compensate for the restricted hypoactivation in the PFC thus producing a disruption in the normal activation network. Further evidence for the inefficiency hypothesis can be observed in the developmentally immature brains of school-age children who show more diffuse, less active and widespread patterns of activation compared to adults during executive tasks (Braet, et al., 2009; Rubia, et al., 2000; Thomas, et al., 1999). An examination from post-mortem work into a structural causal mechanism for DLPFC dysfunction in schizophrenia found that patients had greater neuronal density in the DLPFC than controls (Selemon, Rajkowska, & Goldman-Rakic, 1998). Researchers interpreted the observed increase in neuronal density to be a result of reductions in interneuronal neuropil, which may be one underlying anatomical substrate for PFC dysfunctions in schizophrenia.

Dysfunction of the temporal lobe, specifically in the superior temporal gyrus (STG) has been commonly found in schizophrenia, and has been associated with the experience of auditory verbal hallucinations, such as hearing “voices” (Zhang, et al., 2008). A possible explanation for the reduction in temporal lobe functioning was posited by Heinz and colleagues (2003) who maintained that schizophrenia is related to dysfunction of the temporo-limbic system, usually acquired early in development. They report that after puberty there is a decrease in striatal DA activity which leads to the positive symptoms associated with psychosis.

1.5.4 fMRI and cognitive functioning in individuals at-risk

Between 1992 and the current time of writing a PubMed search revealed 2,733 articles found with keywords “functional magnetic resonance imaging” and
"schizophrenia." Although not every article explicitly tested fMRI in schizophrenia, this superficial search reveals that there is a wealth of functional neuroimaging research into the neural activity which is disturbed in schizophrenia. In contrast, a meta-analysis of fMRI studies of the vulnerability for psychosis studies published from 1992-2006 yielded only 24 studies (Fusar-Poli, et al., 2007). This review revealed that among the studies of the vulnerability for psychosis the most common neurocognitive risk correlates in the brain were localised in the PFC.

Individuals at an elevated genetic-risk for the illness have shown functional abnormalities localised to the DLPFC during neuroimaging tasks of response inhibition (MacDonald, Thermenos, Barch, & Seidman, 2008), working memory (Brahmbhatt, Haut, Csernansky, & Barch, 2006; Callicott, et al., 2003; Karlsgodt, et al., 2007; Keshavan, et al., 2002; Thermenos, et al., 2004), and language processing (Sommer, Ramsey, Mandl, van Oel, & Kahn, 2004). Greater DLPFC activity has been found in unaffected siblings during a content-processing continuous performance task during the low cognitive demanding condition (Delawalla, Csernansky, & Barch, 2008), whereas controls revealed hyperactivity in the DLPFC during the more difficult condition. Whalley et al. (2006) conducted an fMRI study of individuals at a high genetic risk with psychotic symptoms (n= 21) and without psychotic symptoms (n= 41) and controls (n= 21). Results from the fMRI task of verbal fluency revealed that individuals who subsequently developed schizophrenia (n= 4) demonstrated increased parietal lobe and right lingual gyrus activity and decreased ACC activity. A possible explanation for this finding could be the inefficiency hypothesis, which has recently been observed during a neuroimaging study of response inhibition in schizophrenia, that revealed patients recruited widespread fronto-parietal regions to achieve performance levels comparable to controls (Royer, et al., 2009).
A cross-sectional fMRI study of prodromal, first-episode and chronic schizophrenia patients which used a continuous performance visual oddball task found reductions within the VLPFC, ACC, and middle frontal gyrus functioning in the UHR group compared to controls (Morey, et al., 2005). The discovery of a decline in VLPFC functioning before the onset of the illness and in the first-episode and chronic stages represents a potential vulnerability biomarker in assessing risk.

1.6 Diffusion tensor imaging (DTI)

Although there is evidence for functional disconnectivity in schizophrenia, the evidence for structural disconnectivity is less clear. However the recently-developed technique of DTI has for the first time allowed direct examination of white matter (WM) tracts. DTI is an MRI method that quantifies the magnitude and directionality of water mobility (or “Brownian motion”) in three dimensions (Basser, Mattiello, & LeBihan, 1994). At the molecular level Brownian motion is described as the phenomenon of water diffusion in a random fashion in any direction. Moseley et al. (1991) posited that the diffusion of water in the brain reflects tissue architecture, structure, and integrity. When there is a directional dependence of water mobility, the diffusion is described as anisotropic. This anisotropy can be quantified and used to assess the micro-structural properties of the WM, such as organisation of axonal fibres. One measure, fractional anisotropy (FA), is a ratio of the anisotropic component of the entire tensor, which is independent of the degree of anisotropy (Roberts & Schwartz, 2007). A tensor is a mathematical term which describes the relationship between vectors. Highly regular, organised fibres will have greater FA, while less well-organised fibres will have lower FA. Johansen-Berg and Rushworth (2009) have described the biological basis of FA in regards to their corresponding physical characteristics: membrane integrity and conduction time; myelin sheath and
refractory time; axon diameter and probable transmission; packing density and synchronicity of signal. Changes in the corticospinal tract WM have been observed in motor learning and recovery in left hemisphere stroke patients, revealing that positive changes in WM can occur in response to thereapeutic intervention (Johansen-Berg, et al., 2002). The investigation of FA in the at-risk phase, within the context of development, highlights the need to first establish normal age-related developments in WM.

1.6.1 DTI and neurodevelopment

Longitudinal MRI studies of age-related GM and WM changes have revealed that WM shows a linear increase with age, whereas GM volumes in frontal, parietal, and temporal regions reach peak levels during adolescence and decline with age, creating a reduction in the WM-to-GM ratio with increasing age (Bartzokis, et al., 2001; Giedd, et al., 1999; Sowell, et al., 2003). Maturational patterns between the frontal and parietal lobe regions have also been correlated with the patterns of maturation in increased working memory performance and functional activations (Klingberg, O'Sullivan, & Roland, 1997). Normal adolescent maturational WM patterns reveal an age-related increase in FA along the superior longitudinal fasciculus (SLF) (Karlsgodt, Niendam, Bearden, & Cannon, 2009). A study which investigated FA and fMRI analysis in children and adolescents (aged 9-23 years) found that increased FA between the fronto-parietal lobes, which increases with age, underlies less impulsivity during a delayed discounting task (Olson, et al., 2009). In regards to the DLPFC, which has revealed cognitive disturbances in both individuals at-risk and schizophrenia patients, this is last region to undergo GM volume (Gogtay, et al, 2004) and myelination changes (Jernigan & Tallal, 1990). This suggests that synaptic pruning and myelination occur in parallel during young adulthood (Gogtay,
et al., 2004) and therefore grey and white matter differences in this region may not arise and become dateable in this region until brain maturation is complete, around the age of 25.

A recent longitudinal study of adolescents (aged 13-18 years) and young adults (aged 23-42) investigated WM micro-structural changes over time (2.5 year follow-up) (Giorgio, et al., 2008). They found that in adolescent’s age was linearly related to WM increases in FA within extensive regions of the cortex, particularly along the corpus collosum and the corticopsinal tract and associated motor pathways, but also in the cingulum, SLF, inferior fronto-occipital fasciculus, and anterior thalamic radiation. The only longitudinal FA increase observed with age in the young adult group was within the SLF, which still appeared to continue to develop through early adulthood, whereas the other cortical WM tracts plateau. This study is also informative in that they revealed the FA increases were driven by parallel diffusivity, which reflects an increase in the diameter of axon fibres. Therefore, a possible explanation of the underlying changes in micro-structure occurring during adolescence, which are driven by the axon diameter, is believed to reflect the physical property of probable transmission (Johansen-Berg & Rushworth, 2009). The increase in the diameter of axon fibres could also be explained by the calibre of the axon’s volume, which is created by the myelin sheath (Innocenti, Ansermet, & Parnas, 2003). Decreased FA has been interpreted as a loss or alteration in myelin sheath. Disrupted myelination in schizophrenia has been observed in aberrant myelin water fraction in relation to age, suggesting that an absence or delay in maturation has occurred (Davis, et al., 2003; Flynn, et al., 2003).
1.6.2 DTI in schizophrenia

Schizophrenia patients do not seem to reveal this normal pattern of WM and GM development. Instead, patients fail to increase WM with age, therefore a GM deficit is more apparent in younger patients and WM deficits become larger with age (Bartzokis, et al., 2001). These findings of the developmental cortical trajectory are consistent with the synaptic elimination profile, which states that there is prolonged maturation in higher order association cortices, specifically in the prefrontal cortex. Karlsgodt and colleagues (2008, p.1300) have described neurodevelopmental pruning as: a highly regulated process of axonal degeneration, which is mechanistically separate from either axonal retraction or from lesion induced degeneration. The processes that initiate it may include changes in levels and distribution of excitatory and inhibitory neurotransmitters, and changes in the availability of neurotropic factors. Feinberg (1982) has proposed that the onset of schizophrenia occurs when the programmed axonal pruning process and subsequent formation of new pathways that normally take place during adolescence are disturbed.

Douaud et al. (2009) explored longitudinal changes in WM in adolescent-onset schizophrenia patients (age of onset of symptoms between 11-17 years). They found that at baseline the adolescent onset-schizophrenia patients (Mean age: 16 years) had reduced FA along the pyramidal fibre tract, which facilitates motor control, and in the arcuate fasciculus. However, after longitudinal follow-up (Mean age: 18.5 years) the differences in WM disappeared and patients had similar increases in WM to their adolescent control subjects. The initial reductions in FA along the pyramidal fibre tract are important to understanding the neuromotor abnormalities which are found in schizophrenia patients in those at-risk, which have been previously described. Connectivity studies of the pyramidal fibre tract reveals that one-third of its neurons
Chapter One
Introduction to the risk for psychosis

originate in M1 and the rest in premotor and supplementary motor cortices (Nolte, 2002). The tract is associated with the corticospinal tract and the sensori-motor regions falling along it are still developing during adolescence (Paus, 2005; Toga, Thompson, & Sowell, 2006). However, FA reductions in this region reflect a marker of motor skill delays related to WM maturational delays which occur in adolescent-onset schizophrenia, due to the coinciding refinement of motor capacities which occur during childhood and adolescence (Muetzel, et al., 2008).

DTI evidence supporting the disconnection hypothesis shows decreased WM integrity in prefrontal, temporo-parietal, and parieto-occipital regions in schizophrenia (Lim, et al., 1999). The SLF is a fibre tract of particular interest in schizophrenia, serving as the primary connection between the frontal and parietal lobes and its role in supporting working memory functions (Petrides & Pandya, 2002). FA reductions along the SLF have been found in schizophrenia patients (Szeszko, et al., 2008) and at baseline examination in individuals at a high-genetic risk in the medial temporal lobe (Karlgodt, Niendam, Bearden, & Cannon, 2009). Additionally, these reductions have been correlated with verbal working memory performance in first-episode, young adult patients (Karlgodt, et al., 2008b). Lastly, FA reductions in individuals at a high-genetic risk for psychosis have been found within the right parietal lobe (Hoptman, et al., 2008). These findings suggest that the reduction in the parietal region of the SLF observed in the at-risk groups may subserve impairments in working memory that have been found to be a core deficit in schizophrenia (Lee & Park, 2005).

The cingulum fibre bundle is a WM tract of particular interest derived for schizophrenia. Neuroimaging research has revealed that reduced activity in the cingulate during response conflict tasks were coupled with impaired affective responses (Laurens, Ngan, Bates, Kiehl, & Liddle, 2003; Polli, et al., 2008). This
bundle connects the PFC, cingulate gyrus, medial temporal lobe, including the hippocampus and parahippocampus, and precuneus (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005). Studies of FA in schizophrenia patients have revealed that FA along the cingulum is reduced in schizophrenia patients within the PFC (Weiss & Heckers, 2001), cingulate gyrus (Fujiwara, et al., 2007; Kubicki, et al., 2003), and also in the hippocampus in children and adolescents with schizophrenia (White, et al., 2007). A large meta-analysis of FA studies (n=407 schizophrenia patients and n= 383 controls) found the most common reduction in patients occurred in left frontal WM (Ellison-Wright & Bullmore, 2009). A combined fMRI and DTI study of working memory revealed hypoactivity in the PFC, superior parietal lobe, and occipital lobe and FA reductions in the right parahippocampal gyrus and right frontal lobe of schizophrenia patients.

1.6.3 DTI in individuals at-risk

MRI studies of normal brain development during childhood and adolescence have identified this period as a time of rapid neurodevelopment. This developmental period has revealed complex patterns of GM volume loss and increases in WM volume and FA. To date, only one study has examined WM integrity in UHR individuals who are experiencing psychotic-like symptoms. Peters and colleagues (2008) conducted a study examining WM fibre-tracking in UHR subjects (n= 10), first-episode schizophrenia and schizoaffective patients (n= 10) and healthy controls (n= 10). They did not find any WM tract differences between the healthy subjects and the psychosis groups, suggesting that WM abnormalities might not be identifiable in individuals at-risk who experience psychotic-like symptoms. However, this study only examined male participants which are not a representative sample of schizophrenia patients. Also, the lack of WM difference between the schizophrenia
patients and controls is in contrast with previous DTI findings, which have revealed that WM abnormalities are a common and well replicated finding in patients with schizophrenia (see review in Ellison-Wright & Bullmore, 2009).

Previous reports of FA in individuals at-risk have used a genetic-risk approach. Thirty-four pairs of schizophrenia patients and unaffected siblings were examined in a study of FA to determine if specific brain regional patterns of disruption of WM integrity were genetically shared between siblings and probands (Hao, 2009). They found a similar magnitude of reduced FA in the left PFC and the hippocampus in both the siblings and patients, which was significantly different from healthy controls. However, the schizophrenia patients exhibited reduced white matter FA in the left ACC, which was not found in either the relative or control groups. Therefore, left PFC and the hippocampus may be related to higher genetically-mediated risk for developing schizophrenia while WM disruptions in the left ACC may be specific to the presence of the disease. Muñoz Maniega et al. (2008) examined FA in unaffected relatives (n=22) and schizophrenia probands (n=31) and healthy controls (n=51). Lower FA was found in both patients and unaffected relatives in the anterior limb of the internal capsule. This revealed lower FA in fronto-temporal WM which may be a possible genetically-mediated indicator of a higher vulnerability to develop schizophrenia. DeLisi and colleagues (2006) utilised diffusion weighted imaging to investigate the appearance of cortical atrophy in individuals at a high genetic-risk (aged 12-30) along with schizophrenia patients (aged 20-55 years old) and controls. They found an increase in the amount of cerebral spinal fluid (CSF) occupying the interstitial brain space in the region of the left parahippocampal gyrus in both patients and risk subjects. This increase indicates that tissue atrophy may be occurring in this region. Additional FA reductions have also been observed along the SLF at baseline.
examination in individuals at a high genetic-risk (Karlsgodt, Niendam, Bearden, & Cannon, 2009). Another study of young adults at high genetic-risk found focal FA reductions within the left posterior cingulate gyrus, angular gyrus and right precuneus in the at-risk group compared to healthy controls (Hoptman, et al., 2008).

Another approach used in identifying risk for psychosis is to examine genetic variations which may lead to the disruptions in WM in schizophrenia. Winterer et al. (2008) explored the schizophrenia risk gene neuregulin-1 (NRG1), which is involved in neuronal migration, axon guidance and myelination, and a haplotype in the 5' end region of the gene, which is a genetic variation found to double the risk for schizophrenia in an Icelandic population (Stefansson, et al., 2003). They found that reduced medial frontal FA was significantly associated with the NRG1 gene variation. This finding suggests that a single nucleotide polymorphism in a specific region along the NRG1 gene may contribute to the risk for schizophrenia via its impact on myelination in frontal lobe WM. McIntosh et al. (2008) also explored NRG1 and WM, but in unaffected subjects who were genotyped at the NRG1 single nucleotide polymorphism locus. Subjects with the risk-associated genotype had reduced WM volume and FA in the anterior limb of the internal capsule. They therefore provide the first imaging evidence that genetic variation in NRG1 is associated with reduced white matter density and integrity in human subjects. This finding is discussed in the context of NRG1 effects on neuronal migration, axon guidance, and myelination.

### 1.7 Resting-state functional connectivity (RSFC)

Recently, attention has been drawn to fMRI studies reporting findings of ongoing intrinsic activity in the brain in the absence of a functional task (Raichle & Mintun, 2006). RSFC is a relatively new technique which has been advancing the understanding of the complex relationships between distributed brain regions.
subserving cognition. Studies of RSFC examine the correlations in slow spontaneous fluctuations (<.1 Hz) in the BOLD signal. Biswal and colleagues (1995) were the first to report spontaneous activation fluctuations in the BOLD signal from one region of the motor cortex to other parts of the same functional network when the participant was at rest and not performing a task.

The system of synchronised task-related reduction, known as the task-negative, network, which is suspended during goal-directed behaviours has been termed the “default-mode network” (DMN) of the brain (Raichle, et al., 2001). The DMN is conceptualised as a stimulus-independent network of low frequency oscillations that decrease during task-related activity and increase during self-monitoring and reflection (Gusnard, Akbudak, Shulman, & Raichle, 2001).

1.7.1 RSFC in schizophrenia

An electroencephalography (EEG) study of resting-state in first-degree relatives of schizophrenia patients (Venables, Bernat, & Sponheim, 2009) found increased high-frequency oscillations (beta) in frontal regions in both patients and unaffected relatives. However, a further investigation of genetic liability found that only schizophrenia patients exhibited increased low-frequency EEG activity (delta and theta), which was related to the presence of a Val158 Met polymorphism on the COMT gene. Another resting-state EEG study in unaffected relatives also found cortical hyperactivation during beta-activity and a similar trend in probands, additionally, EEG coherence data in the temporal lobes was found to be reduced in both groups (Winterer, et al., 2001). These findings indicate that excessive high-frequency beta waves, which are actively engaged during concentration, and reduced neuronal synchronization during delta frequency in temporal lobes, provides possible endophenotypes derived from resting-state data. FMRI studies of the RSFC of the
DMN in schizophrenia patients are limited but have revealed RSFC abnormalities in patients, however the studies reported are less than consistent in their findings (see review in Greicius, 2008).

Liang and colleagues (2006) were the first to systematically study resting-state fMRI in schizophrenia. They found widespread differences in connectivity between patients and controls, which were predominately decreases but also included increases in connectivity between regions in patients. Others studies of RSFC in schizophrenia patients have identified reduced connectivity originating in empirically derived seed regions of interest (ROI) (Bluhm, et al., 2007; Hoptman, et al., 2009; Zhou, et al., 2008). Bluhm et al. (2007) found reduced connectivity between the posterior cingulate and other DMN regions in schizophrenia patients. Hoptman et al. (2009) discovered disruptions in schizophrenia patients during the resting-state between the amygdala and ventral PFC, ACC, insula connectivity and also between the lentiform nucleus with orbitofrontal cortex and DMN regions. Zhou et al. (2008) examined RSFC and FA in schizophrenia patients and found reduced connectivity in patients between the anterior hippocampus and DMN regions. They also found that reduced parahippocampus FA which was accompanied with FA reductions in the fornix in patients. Additionally, in a study of elderly patients with a mild cognitive impairment, who are at an elevated risk for Alzheimer’s Disease, reductions were found between hippocampal RSFC and DMN regions (posterior cingulate and medial PFC) (Sorg, et al., 2007). The studies of hippocampal functional connectivity reveal a region that may potentially underlie the memory impairments observed in the disorganised symptoms exhibited in schizophrenia patients.

Only three studies have reported increased RSFC in schizophrenia patients (Jafri, Pearlson, Stevens, & Calhoun, 2008; Pomarol-Clotet, et al., 2008; Zhou, et al,
Regions in the DMN and also in frontal, basal ganglia, parietal, and occipital lobes have revealed increased connectivity in patients (Jafri, Pearlson, Stevens, & Calhoun, 2008). The only reductions in the patient’s RSFC were between the temporal and parietal lobes. A study which investigated a seed ROI in the DLPFC, revealed hypoactivity in schizophrenia patients during a working memory task, revealed increased connectivity with the DMN (medial PFC) (Pomarol-Clotet, et al., 2008). Together, findings indicate failures to recruit and activate task-positive regions during cognitive tasks and deactivate task-positive networks during rest. Lastly, Zhou and colleagues (2007b) reported increased correlations of the RSFC in patients in both the DMN (medial PFC, posterior cingulate, precuneus, left parietal cortex, and inferior temporal gyrus) and in the task-positive networks (right DLPFC, supplementary motor area, and bilateral orbitofrontal gyrus). Increased connectivity within both these networks demonstrates an increased sensitivity in patients for endogenous and exogenous stimuli. Therefore, with both networks co-activating this could result in excessive competition for neural resources to carry out both goal-directed behaviours and monitor internal states.

Studies examining the relationship between symptoms which have been shown to be involved in cognitive processing and RSFC have also been investigated. Negative symptom correlations have been investigated in schizophrenia through the use of positron emission tomography (PET) during resting-state scans. A negative correlation was found between increased physical anhedonia scores and reductions in the medial PFC (Park, et al., 2009). Additionally, symptoms ratings from the Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein, & Opler, 1987) revealed that negative symptom scores were positively correlated with increased resting-state activity in the cerebellum. Therefore, the comprised functional connectivity networks
described in patients could give rise to reduced integration in cortical synchronicity across the entire brain (Bressler, 2003).

There have only been two reports of RSFC in first-episode patients to date (Lui, et al., 2009; Zhou, et al., 2007a). Zhou and colleagues (2007a) investigated RSFC in medicated first-episode patients and found a negative correlation in RS and activations in a DLPFC seed between the posterior cingulate, posterior parietal cortex, thalamus, and striatum. However, the DLPFC seed revealed increased connectivity with the left middle temporal gyrus and paralimbic regions in patients. Lui and colleagues (2009) used seed ROIs derived from their findings of GM reductions in the three cortical regions in the antipsychotic-naïve first-episode schizophrenia patients and correlated RSFC in these regions with symptom scale scores. Increased RSFC in the middle temporal gyrus seed and putamen revealed a positive correlation with PANSS thought disturbance scores. However, a decrease between connectivity of DMN regions (middle temporal gyrus seed and precuneus) revealed a positive correlation with negative and anergia scores. The findings of the increased connectivity with the middle temporal gyrus and DLPFC and putamen may reveal a vulnerability marker present at illness onset.

RSFC analyses are also gaining a validity to provide direct measurements of neural networks which may underlie the extent of neurocognitive deficits which are observable in schizophrenia patients and will hopefully shed new light on the identification of disruptions in functional integration which are precursors of the illness. To date there are no published reports of RSFC in individuals at-risk for psychosis. The investigation presented in Chapter Four, will be the first report of resting-state fMRI in individuals at a heightened risk for psychosis, contributing new
insights into the aberrant neural pathways subserving cognition in individuals vulnerable to developing schizophrenia.

1.8 Summary of chapters

It is becoming evident that elucidation of the aetiology of schizophrenia will likely require a multi-modal approach to the study of brain structure and function within the context of development. Examining vulnerability through the use of multiple neuroimaging techniques I seek to explore the theories of neurodevelopment and disconnectivity which underlie the pathophysiology leading to the development of schizophrenia. Empirical Chapters Two, Three, Four and Five each describe MRI investigations into understanding the neural underpinnings of the vulnerability for developing psychosis. Appendix One reports a novel analogue to a well-known neuropsychological test which was created for fMRI and lends itself to detecting the neural underpinnings of impaired set-shifting abilities in schizophrenia patients. Appendix Ten reports preliminary data on a future longitudinal study I am carrying out to assess ventral striatum functioning in adolescents at-risk for psychosis.

Chapter Two explores the neural correlates of the vulnerability stage of psychosis through investigating children who are experiencing psychotic-like symptoms. A population-based study, entitled the Adolescent Brain Development (ABD) Study, was conducted among school children to identify children who may be at an elevated risk for developing psychosis, based on the previous work of Poulton et al. (2000). Children were identified as at-risk, based on a priori criteria scores of the Adolescent Psychosis Screener (APSS). The at-risk and control children, who did not have psychotic-like symptoms, were invited for a clinical interview to explore further any psychotic symptomatology. After children were identified as in either the at-risk or control groups a neuropsychological testing session (results reported in Appendix
Five) took place. The next session included the neuroimaging portion of the study. This session comprised a multi-modal investigation which assessed brain function using an event-related response inhibition task, brain structure assessed through an optimised VBM analysis, and WM structure assessed through FA from DTI scans. Findings of widespread reductions in FA would support the disconnection hypothesis of schizophrenia, which suggests that behavioural abnormalities in schizophrenia can arise from dysfunctional neuronal integrations which are altered through subtle micro-structural abnormalities. My investigation combines structural and functional results in individuals at-risk and compares them to normally developing children without psychotic-like symptoms.

Chapter Three examined a group of chronic schizophrenia patients in the chronic schizophrenia (ChSz) study with the same multi-modal measures used with the at-risk children in Chapter Two. Between-subjects results are presented for the tMRI, VBM and DTI results. Additionally, in this chapter, I present a combined group analysis which pooled the data of the schizophrenia patients and at-risk children into one group (PSY) and the adult and children controls (CON) to determine the patterns of functional and structural abnormalities found in both psychosis groups, independent of the stage of the disease data. The functional and structural abnormalities found in common in the PSY group may reveal early biomarkers related not only to the vulnerability for developing psychosis but persistent in the underlying pathology present in those who have developed the disease.

Chapter Four examines the inter- and intra-hemispheric connectivity disturbances, which has been suggested to play a major role in the development of schizophrenia (McGlashan & Hoffman, 2000; Stephan, Baldeweg, & Friston, 2006). RSFC was collected in both the ABD and ChSz studies and was analyzed in parallel.
Seed regions derived from the functional and VBM analyses were used as ROIs to probe task-related regions of synchronous activity during the resting-state.

The early detection of psychosis is paramount to reducing the effects of untreated psychosis. Studies of the duration of time of untreated psychosis have revealed that the longer this period the poorer the outcome is for the patient. The work presented in this thesis aims to identify the earliest vulnerability markers in the brain which stem from findings of cognitive, functional, structural, and connectivity methods of investigation.
2. Chapter Two
The Adolescent Brain Development study:
Structural and functional brain correlates of subclinical psychotic symptoms in 11-13 year old schoolchildren

Acknowledgement of authorship for this chapter:

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2.1 Abstract

Studying children experiencing psychotic symptoms provides a unique opportunity to examine the vulnerability to psychosis within the context of development. Using neuroimaging techniques this study investigated cognitive control functions, brain volumetrics and white matter (WM) integrity in an at-risk cohort of children. Between-subjects assessment of brain function and structure among 11 school-going, non-treatment seeking children aged 11-13 years old who were at symptomatic risk for psychosis (AR) and 14 healthy control children aged 11-12 years old without subclinical psychotic symptoms (CON). MRI assessments included: Functional measures of response inhibition and error-related processes, whole-brain VBM of grey matter (GM) and DTI utilising fractional anisotropy (FA) to probe WM integrity. FMRI results showed reduced activity in the AR group within right frontal and bilateral temporal cortex for response inhibition and reduced activity within the anterior cingulate, insula, and middle frontal gyrus for error-related processing \( (p<.05, \text{ corrected}) \). VBM analysis revealed GM increases in the AR group within middle and superior temporal gyri, angular gyrus, orbitofrontal gyrus, and GM decrease within the inferior temporal gyrus \( (p<.05, \text{ corrected}) \). DTI analysis identified WM decreases in the AR group along the inferior fronto-occipital fasciculus, cingulum and inferior longitudinal fasciculus \( (p<.05, \text{ corrected}) \). This multi-modal investigation revealed aberrant fronto-temporal dysfunction, coupled with increased fronto-temporal-parietal GM and reduced temporo-limbic-occipital WM integrity, which provide potential early neurocognitive risk biomarkers related to the susceptibility for developing psychosis and subsequently the neurodevelopmental trajectory leading to schizophrenia.
2.2 Introduction

Schizophrenia is a severe mental disorder that typically has its onset in late adolescence or early adulthood. It has long been evident that the disease process begins much earlier, as found in longitudinal studies of childhood development, which have shown individuals who later go on to develop schizophrenia show motor, language, and cognitive impairments during infancy (Cannon, et al., 2002b; Cannon, et al., 2003; Jones, Rodgers, Murray, & Marmot, 1994). Pre- and perinatal complications have been consistently shown to increase the risk of later developing schizophrenia (Clarke, Harley, & Cannon, 2006) and are likely to interact with genetic risk factors (Mittal, Ellman, & Cannon, 2008). These pre- and perinatal disturbances may interfere with fundamental processes such as the development of the brain’s neural circuits. Later development of schizophrenia is also susceptible to environmental risk factors that increase the risk for psychosis such as cannabis use and childhood trauma (Arseneault, Cannon, Witton, & Murray, 2004; Kelleher, et al., 2008). The exploration of how these risk factors influence normal developmental processes is an important target of future research. Taken together, these findings emphasise the importance of studying the vulnerability to psychosis within the context of development.

2.2.1 Neuroimaging in schizophrenia

Imaging studies have established that schizophrenia is associated with structural brain abnormalities, notably enlarged ventricles and reduced temporal and PFC WM volumes (Shenton, Duckey, Frumin, & McCarley, 2001; Wright, et al., 2000). A “back-to-front” wave of cortical grey matter (GM) loss has been noted in participants with childhood-onset schizophrenia compared with age-matched controls (Thompson, et al., 2001). GM volume deficits have also been found in individuals at-
risk for schizophrenia (Job, Whalley, Johnstone, & Lawrie, 2005) and around the time of transition from prodromal state to psychosis (Pantelis, Yiicel, Wood, McGorry, & Velakoulis 2003) indicating that structural and functional abnormalities are present, at least in part, before the onset of the illness and are likely to be linked to disruption of normal brain developmental processes.

In contrast to structural brain imaging studies there is sparse information on fMRI abnormalities prior to the onset of psychosis (Morey, et al., 2005) or in the at-risk state (Whalley, et al., 2004). Diffusion tensor imaging (DTI) is a relatively new magnetic resonance imaging technique allows for detailed analysis of WM fibre tract integrity. This analysis has the ability to assess structural connectivity among brain regions that have shown reductions amongst individuals with schizophrenia. One tract in particular showing reduced fractional anisotropy is the uncinate fasciculus (UF), a tract connecting the frontal and temporal lobes (Kubicki, et al., 2002). This technique is now beginning to be used to investigate WM changes during the prodromal stage (Karlsgodt, Niendam, Bearden, & Cannon, 2009; Walterfang, et al., 2008).

2.2.2 Neuroimaging individuals at-risk for schizophrenia

Historically, risk for schizophrenia was approached from the point of view of familial risk by studying relatives with an affected first-degree family member (Cornblatt, Obuchowski, Roberts, Pollack, & Erlenmeyer-Kimling, 1999; Johnstone, Lawrie, & Cosway, 2002). The aim of this genetically mediated approach is to define intermediate phenotypes of psychosis, in particular using a functional neuroimaging approach, to probe cognitive functioning during tasks that show impairment in those with the established illness and those with a genetic predisposition. Functional abnormalities have been shown amongst first-degree relatives during tasks of response inhibition (Vink, Ramsey, Raemaekers, & Kahn, 2006), language processing
(Li, et al., 2007; Sommer, Ramsey, Mandl, van Oel, & Kahn 2004), working memory (Brahmbhatt, Haut, Csernansky, & Barch, 2006; Callicott, et al., 2003; Karlsgodt, et al., 2007; Keshavan, et al., 2002; Thermenos, et al., 2004), verbal declarative memory (Thermenos, et al, 2007; Whyte, et al., 2006), and theory of mind (Marjoram, et al., 2006). The commonality underlying these risk studies is a dysfunction of the PFC; however, results vary with regards to some studies reporting hypoactivity and others hyperactivity within the PFC in patients and relatives. Hypoactivity has been attributed to participants performing poorly on a task, in which the demand of the task exceeds the participant’s abilities and is evidenced by reduced activity within the DLPFC (Callicott, et al., 2003). Weinberger and McClure (2002) have posited that hyperactivity is a result of inefficient and less automatic processing within the PFC when the participant is performing well on a task and is evidenced by extraneous neural activity. Results from familial investigations continue to provide attractive intermediate phenotypes related to a genetically transmitted risk for the illness.

2.2.3 Identifying symptomatic-risk for psychosis

More recent approaches have used an ultra high risk approach which involves identifying individuals in late adolescence who are seeking treatment for prodromal symptoms of psychosis (Cannon, et al., 2008; McGorry, et al., 2002). An alternative high-risk approach involves studying children at symptomatic risk of psychosis, that is, children who report subclinical psychotic symptoms. A number of findings have demonstrated a continuum between the non-pathological subclinical phenotype and clinical psychosis, including shared risk factors such as reduced IQ (Horwood, et al., 2008), ethnicity (Laurens, West, Murray, & Hodgins, 2008), and childhood trauma (Kelleher, et al., 2008). Longitudinal research has shown that children who report subclinical psychotic symptoms are at an increased risk, ranging from 16-to 65-fold
increase, to later develop a clinical psychotic disorder (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005; Poulton, et al., 2000). In a New Zealand birth cohort (Poulton, et al., 2000) 11-year olds who reported psychotic symptoms were at 5- to 16-fold increased risk of adult psychotic illness. Twenty-five percent of the children who displayed strong, definite symptoms at age 11 were diagnosed with a schizophrenia-spectrum disorder by age 26. A population-based study in the Netherlands (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005) in adults aged 18-64 found that the 2-year transition rate to clinical psychotic disorder was more than 65-fold higher for those who had previously reported subclinical psychotic symptoms compared to those without prior psychotic-like experiences. The two-year risk rose to 21% for those with multiple psychotic experiences, and to 15% for those whose psychotic experience had arisen in the context of significant lowering of mood. In a general population in Munich, Germany (Dominguez, Wichers, Lieb, Wittchen, & van Os, 2009), a sample of 845 adolescents, aged 14–17 years, were assessed for psychotic symptomatology four times over a period of eight years. They found that that subclinical psychosis was confirmed to be common, occurring in 22% of the sample but was mostly transitory (i.e. only occurred once or twice), with recurrence or persistence occurring in 30–40% of those with symptoms. Additionally, of those diagnosed with psychosis at the final intake (n= 47), 38% had symptoms that were identifiable in the first three intakes, and 20% had symptoms that occurred more frequently. Therefore, clinically relevant psychosis in young adults could be traced to the subclinical psychosis phenotype expressed up to eight years earlier, with the occurrence of the symptoms to be a strong indicator of those whose symptoms will persist.

There is some controversy surrounding the identification of this risk group given the high proportion of adults who endorse psychotic-screening questions.
Approximately 28% of the individual’s from the U.S. National Co-morbidity Survey admit experiencing a psychotic-like experience (i.e. hearing voices at least once a month), however when interviewed by a clinician there was only a 0.7% rate of psychosis diagnosed in that subgroup (Kendler, Gallagher, Abelson, & Kessler, 1996). Other studies report anywhere between 4-71% of participants have a lifetime incidence of a hallucinatory experience (Barrett, 1992; Johns, Nazroo, Bebbington, & Kuipers, 1998; McKellar, 1968; Posey & Losch, 1983; Poulton, et al., 2000; Tien, 1991; van Os, Hanssen, Bijl, & Ravelli, 2000; van Os, Linscott, Myin-Germeys, Delespaul, & Krabbendam, 2009).

To investigate this phenomenon Kelleher and colleagues (2009) tested the validity of the psychosis screening instrument used in the present study, the Adolescent Psychotic-Like Symptom Screener (APSS). They found 38% of the total sample (aged 11-13 years old) endorsed at least one item on the seven-item psychosis screening questionnaire. They found that when a single question regarding the presence of auditory hallucinations (“Have you ever heard voices or sounds no one else can hear?”) was endorsed and verified to be present during a follow-up clinical assessment this question attained a positive predictive value of 71% and a negative predictive value of 90%. Furthermore, this questionnaire’s positive predictive value rose to 100% when any psychotic-like experience was endorsed during the clinical assessment (i.e. Grandiose or paranoid symptoms). This validation of the APSS shows that this quickly administered screening tool has the opportunity to become an economical and standardised screening instrument for psychotic symptomotology in the school-age population. Therefore, although there is a high prevalence of psychotic-like symptoms amongst the general population, our research has systematically identified individuals through the use of the clinically validated APSS
to reduce the instance of false positives that arise when conducting population-based research.

2.2.4 Response inhibition and schizophrenia

Response inhibition refers to the suppression of actions, responses, stimuli, thoughts, memories, or learned ways of performing a task that are inappropriate in a given behavioural context or that are unwanted because they interfere with the completion of a motor or cognitive task (Fassbender, et al., 2004). The ability to inhibit inappropriate mental states, such as distracting endogenous and exogenous stimuli or inappropriate motor responses, is essential in executing goal-directed behaviours. Specifically, DLPFC and ventral lateral prefrontal cortex (VLPFC) regions have been commonly found to be the central regions activated during response inhibition (Blasi, et al., 2006). A meta-analysis of GO/Nogo tasks, which required the participant to suppress a pre-potent response, found prominent activation patterns in the right frontal cortex during successful inhibitions (STOPS) (Buchsbaum, Greer, Chang, & Berman, 2005). A separate meta-analysis of the “XY” GO/Nogo task (Figure 2.1), which is the task used in the current study, found right DLPFC and VLPFC activations for successful inhibitions (Garavan, Hester, Murphy, Fassbender, & Kelly, 2006).

Response inhibition in schizophrenia patients have revealed activation reductions in the DLPFC and ventrolateral prefrontal cortex (VLPFC) (Ford, et al., 2004; Kaladjian, et al., 2007; Kiehl, Smith, Hare, & Liddle, 2000; Minzenberg, Laird, Thelen, Carter, & Glahn, 2009; Rubia, et al., 2001; Weisbrod, Kiefer, Marzinzik, & Spitzer, 2000). Neuroimaging studies examining other response inhibition tasks in schizophrenia patients have also indicated a deficit in the PFC during Stroop tasks (Barch, Carter, Hachten, Usher, & Cohen, 1999; Henik, et al., 2002), context-
processing tasks (Javitt, Shelley, Silipo, & Lieberman, 2000) and also in unmedicated first-episode patients during context-processing (Barch, et al., 2001).

Figure 2.1. The experimental design of the GO/NOGO XY task. A serial stream of the letters X and Y at the rate of 1 Hz were presented during the task. Participants were instructed to make a one-button press when each letter was presented on the screen. These trials are labelled as the “Standard” and are known as the “Go” trials. For the trials in which the same letter which was presented in the previous trial was repeated participants were instructed to withhold the button press response. These trials are labelled as “Lure” and are known as the “NoGo” trials. During the task there were 450 “Go” trials and 50 “NoGo” trials.

2.2.5 Cognitive functioning in individuals at-risk

An interest in studying neurobehavioural functioning during early development to aid in the prediction of the vulnerability for psychosis began in 1952 with the prospective, longitudinal work of Fish (1987). In this seminal study infants who were genetically at-risk (i.e. offspring of mothers diagnosed with schizophrenia) (n= 12) were followed from one-month of age until age 20/22. They found that the individuals who later developed a psychotic disorder (n= 7) had social-affective and
perceptual-cognitive deficits during development which were detectable between two and six years of age.

The Jerusalem Infant Study (Hans, et al., 1999) was another prospective, longitudinal study which began in 1973 and studied infants who were the offspring of a parent with schizophrenia and also infants who had a parent diagnosed with a non-schizophrenic mental disorder. During the follow-up of neurobehavioural functioning during childhood and adolescence researchers found that 42% (n= 10/19) of the offspring of a parent with schizophrenia had poor neurobehavioural functioning at both follow-up phases, particularly motor and cognitive functioning, compared to 22% (n= 5/17 from the other mental illness and n= 4/11 no mental illness groups). This study revealed that offspring of schizophrenia patients were three times more likely to have deficits in neurobehavioural functioning as offspring on non-schizophrenia parents and that these deficits remained stable over time.

Another longitudinal study was the New England Birth Cohort (Seidman, et al., 2000) which began in 1959 studied individuals who had a prenatal or perinatal complication. Participants were studied at age seven and examined the relationship of neurocognition to obstetric complications, which included: low birth weight, probable hypoxic ischemic complications, and chronic hypoxia. At follow-up at age seven (n= 11,889) cognitive measures were assessed and researchers found that all three pre- and perinatal complications were associated with lower neurocognitive performance, especially in the domains of perceptual-motor abilities, academic achievement skills, and verbal-conceptual abilities, therefore revealing how pre- or perinatal insults can effect neurodevelopment and subsequent cognitive performance.

Two other studies, the Harvard Adolescent High-Risk and Hillside Family studies (Seidman, et al., 2006), examined not only offspring of persons with
schizophrenia but also siblings of schizophrenia (n=73) and affective psychosis patients (n=18) and controls (n=84). Participants (age range 12-25 years old) were compared on overall neurocognitive functioning in six domains. The relatives of the schizophrenia patients showed significantly lower verbal ability and working memory than controls. The relatives of affective psychosis patients showed reduced verbal ability compared to controls. They found that low IQ (<95) was not specific to offspring of schizophrenic parents; however, impairments in attention were found to be the best cognitive link to a genetic predisposition to schizophrenia. The specificity of premorbid IQ was explored by Woodberry et al. (2008) who conducted a meta-analysis of 66 studies of premorbid IQ in psychosis patients from the last 50 years and found that a decline in IQ scores pre- and post-onset of psychosis was related to the onset of frank psychosis. However, IQ does not seem to be the most specific variable related to the vulnerability for schizophrenia and more recent studies have examined more aspects of neurocognitive functioning in individuals at-risk.

Brewer et al. (2006) described the neurocognitive antecedents to the development of psychosis in a review of studies which used different identification approaches to studying individuals at-risk. The main focus of the review emphasised cognitive functioning in ARMS individuals who later converted to psychosis and suggested specific olfactory identification and spatial working memory deficits exist prior to illness onset. Studies from Brewer and colleagues (1996; 2005) have demonstrated deficits in high-risk individuals in the cognitive domains of attention, memory and executive functioning, which were intermediate between performance in first-episode patients and controls. Impaired olfactory identification was also a baseline deficit which was detectable in ARMS individuals before their transition to the illness (Brewer, et al., 2003). Other studies of individuals in the ARMS who later
converted to psychosis exhibited performance deficits at baseline that declined at a
12-month follow-up on a visual reproduction memory test and the Trail-making Test
B condition (Wood, et al., 2007) and a verbal memory task (Lencz, et al., 2006). A
retrospective study of prodromal individuals revealed impaired selective attention
abilities during continuous performance executive processing tasks which were
detectable before illness onset (Cornblatt, et al., 2003).

A longitudinal study (Hambrecht, et al., 2002) of individuals reporting
psychotic symptoms (n=29) revealed that verbal fluency was impaired relative to
controls, but this cognitive variable was not predictive of conversion. A large
retrospective longitudinal birth cohort study (n=1037) (Cannon, et al., 2002a), which
assessed neurocognitive functioning in individuals at age 11, revealed that deficits in
neuromotor functioning, receptive language, and cognitive development were
predictive of the conversion to a psychotic illness at age 26. In Appendix Five, a
manuscript (submitted) that I co-authored, is presented which details the neuromotor
and receptive language deficits in my sample of at-risk children. A study of UHR
individuals (Niendam, et al., 2006) aged 12-35 years old also found deficits in motor
speed and speed of processing tests, which included the Trail-Making Form B test, in
UHR individuals compared to normative samples. Laurens and colleagues (2007)
compared children aged 9-12 years old who were experiencing psychotic-like
symptoms to children free of psychotic-like symptoms. They found that children who
meet all three of the following criteria were at the highest risk: caregiver reports of
abnormal speech or motor development in the child; caregiver reports of social,
emotional, or behavioural problems in the child; and child reports of psychotic-like
experiences, revealed worse performance on cognitive tests, had worse performance
on verbal working memory and response inhibition tasks. They concluded that these
Chapter Two

ABD study

deficits are less pronounced than those seen in a comparison first-episode group of patients, but appear similar in magnitude to those found in young treatment-seeking prodromes. A recent event-related potential (ERP) study, which implemented the same identification criteria of children at-risk reported in this thesis, found that children at-risk seem to show a different pattern of processing language than age-matched healthy control children (Personal correspondence with M. Cannon), which supports our finding of receptive language deficits. Crow (1990, 1995) has stressed the role of a genetic predisposition to the presence of language disturbances, which are subserved by an inability to form normal brain asymmetries. Together these data suggest that neurocognitive deficits which are identifiable in the childhood and adolescent at-risk stages, specifically point to neuromotor, receptive language, verbal working memory, speed of processing (Trail-Making Test Form B), IQ score, and sustained and selective attention as predictive of a later conversion to psychosis. Lauren’s finding of behavioural deficits during response inhibition in children with psychotic-like symptoms is further explored through my fMRI study of response inhibition in children at-risk.

The experience of subclinical psychotic symptoms appears to index individuals at increased risk for later psychotic disorder and provides an opportunity to study the developmental trajectory to psychosis. I hypothesise that the children found to be at-risk will show functional and structural dysfunctions, particularly within prefrontal and temporal regions. The disconnection syndrome has been developed within the context of schizophrenia by Friston and Frith (1995) to describe the disruption of prefronto-temporal communication based on empirical data from functional neuroimaging. The aim of the present study was to use a multi-modal imaging approach to investigate response inhibition functioning and anatomical
neural variation among a young at-risk group compared to controls without symptoms to probe the hypothesis of frontal-temporal dysfunction.

2.3 Materials and methods

2.3.1 Participants

The study was approved by the Beaumont Hospital Medical Research Ethics Committee and the Trinity College School of Psychology Research Ethics Committee. Following a complete description of the study, which was given to the child and their parent all participants and their parents gave written consent to participate (see Appendices Three, Six and Seven). Children with subclinical psychotic symptoms, termed the at-risk group (AR), and comparison children with no subclinical symptoms, termed the control group (CON) were recruited from primary level schools in Dublin, Ireland following screening in the classrooms using a short seven-item APSS screening instrument (Figure 2.2). The APSS questionnaire included four of the five questions used in the New Zealand birth cohort study (Poulton, et al., 2000) which were derived from the Diagnostic Interview Schedule for Children. We also included three additional questions to assess visual hallucinations, delusions of control and grandiosity. The questionnaire was scored as follows: Yes, 1 point; Maybe, 0.5 point; No, 0 points.

There were a total 277 children who completed the APSS questionnaire in the classroom. Total scores were calculated for each participant by summing the scores from each of the seven questions. Initial inclusion criteria into the at-risk group were based on the a priori decision that an individual had to have a total score of two or more on the screening instrument. Individuals with a total score of less than two (the potential control group) and those who scored two or more (the potential at-risk group) on the questionnaire were invited via a letter sent to their home to take part in the
Please tick one box for each question

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<td>Some people believe that their thoughts can be read by another person. Have other people ever read your mind?</td>
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<td>Have you ever had messages sent just to you through TV or radio?</td>
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<td>Have you ever thought that people are following you or spying on you?</td>
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**Figure 2.2 The seven-item Adolescent Psychotic-Like Symptom Screener (APSS).**

A self-report questionnaire, administered during the classroom session, to screen for the presence of psychotic symptoms.

Clinical interview. On subsequent interview some of the individual’s in the at-risk group were found not to have any symptoms present and were therefore excluded from the at-risk group and included in the control group. Other exclusion criteria from the study included age >11 or <13 years, non-fluent English speakers, non-Caucasian ethnicity, neurological disorder, mental disability, and IQ lower than 70. Individuals with a family history of psychosis or pre-perinatal complications were not excluded from the study.

The APSS proved to be a good screening indicator of psychotic symptom severity, for example, children with the two highest APSS score were confirmed to be AR during the clinical interview. Also, among the interview confirmed AR participants all but one AR individual had scored above a total score of one and also endorsed either visual or auditory hallucinations on the APSS. The total APSS scores, out of a possible seven, significantly differed between groups (CON: 1.0±1.4 points, range: 0-4, AR: 3.0±1.1, range: 1-5 points; t(23)=4.0, p=.001). A study which validated the usage of the APSS (Kelleher, et al., 2009) in an expand sample of the participants presented in this chapter (n= 334 vs. n= 277) found that 82% of the at-
risk group, verified at interview, had met a score of greater than two on the APSS. Additionally, the at-risk selection criteria of scoring a total of two or more correctly identified participants with subclinical psychotic symptoms during the time of interview with 70% sensitivity and 83% specificity.

2.3.2 Assessments

Children who took part in the imaging study underwent a clinical interview with a trained psychiatrist or psychologist to verify the presence or absence of psychotic symptoms. There were a total of 37 children whose parents gave consent and participated in the clinical interview, which were classified post-interview to be 20 CON and 17 AR children. Not all of these subjects interviewed completed the imaging section of the study due to reasons such as: lack of consensual group classification, personal choice not to be scanned, did not complete the entire scan, and contra-indications to scanning. The interview schedule used in this study was the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime version (K-SADS) (Kaufman, et al., 1997) and the Structured Interview for Prodromal Syndromes (SIPS) (McGlashan, Miller, Woods, Hoffman, & Davidson, 2001). Both the children and one of their parents were interviewed. During the K-SADS parent/caregiver interview they were asked specific questions about any prenatal or birth complication and also specifically about the child’s family history of medical and psychiatric illnesses. The Psychosis section of the K-SADS assessed both current and past psychotic-type experiences with positive responses prompting detailed questioning and administration of the detailed Psychosis supplement of the K-SADS. All interview transcripts were reviewed by a consensus diagnostic meeting comprising psychiatrists and psychologists to judge whether a participant had experienced a “definite” psychotic symptom, such as an auditory hallucination,
varying in frequency from a couple of discrete events to several times a week. Global functioning was assessed with the Children’s Global Assessment Scale (CGAS) (Shaffer, et al., 1983). Participants also completed a test of general scholastic ability (WRAT-4) (Wilkinson & Robertson, 2006).

2.3.3 Functional magnetic resonance imaging (fMRI) task

Participants completed a GO/NOGO task during functional imaging. Stimuli consisted of a 1 Hz serial stream of alternating X and Y letters which were presented in white against a black background. Each letter was presented for 600ms and followed by a blank, black screen for a 400ms fixed interstimulus interval. Participants were instructed to press a button for each stimulus while it was still present on the screen. NOGO stimuli, in which the target stimuli did not alternate (e.g., the fifth letter in the following sequence: X, Y, X, Y, Y, X…) required inhibition of the response (Figure 2.1). There were 450 GO and 50 randomly distributed NOGO stimuli presented over two runs.

2.3.4 Imaging acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a 30.5 cm internal diameter three-axis local gradient coil with insertable radiofrequency coils with transmit–receive capabilities. Participants viewed stimuli back-projected onto a screen at the end of the scanner bed viewed through prism glasses. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV=230 mm, thickness 0.9 mm, voxel size 0.9 mm × 0.9 mm × 0.9 mm) were acquired. Functional data were collected using a T2* weighted EPI sequence that acquired 32 non-contiguous (10% gap) 3.5mm axial slices covering the entire brain (TE=35ms, TR=2000ms, FOV=224 mm, 64 mm × 64 mm matrix size).
At the beginning of the functional scan four discarded acquisition scans (8s) were taken to allow for the stabilisation of T1 effects on the BOLD signal. Diffusion-weighted images were obtained using spin echo EPI (TE=52ms, TR=12343ms, FOV=224 mm, 150 mm matrix size) with 15 isotropically distributed orientations for the diffusion-sensitizing gradients at a $b$ value of 800 s/mm$^2$ and one $b=0$ image. All scans were corrected for movement and distortion correction using successive affine registrations before being manually pre-processed.

2.3.5 fMRI analysis

Imaging data were analyzed using the AFNI (Cox, 1996) software package. Following image reconstruction, the two 3-D time series (runs one and two) were concatenated, then motion-corrected to the fifth volume of the first functional run using 3-D volume registration (least-squares alignment of three translational and three rotational parameters) and edge detected. In regards to excluding subjects with excessive movement, which could not be corrected for during the motion correction process, an a priori threshold of >3mm of movement was used for exclusion of participants. However, no participant in this study needed to be removed for excessive movement due to meeting our movement exclusion criteria. A deconvolution analysis calculated the hemodynamic response functions for successful inhibitions (STOPS) and errors of commission (ERRORS) and these were then modelled using a gamma-variate function. The modelling, using nonlinear regression identified the best fitting gamma-variate function for each voxel. The area under the curve of this hemodynamic model was calculated for each voxel and expressed as a percentage of the area under the baseline (representing tonic task-related processes). These activation maps were resampled at 1 mm x 1 mm x 1 mm resolution, then warped into
standard Talairach space and spatially blurred with a 4.2 mm full width half-
maximum (FWHM) isotropic Gaussian kernel.

Separate AR and CON activation maps for each trial type (STOPS and
ERRORS) were determined with one-sample *t*-tests against the null hypothesis of
zero activation change. Significant voxels passed a voxelwise statistical threshold

\[ \text{CON: } t(13)=3.37, p<.005; \ AR: t(10)=3.58, p<.005 \]

and were required to be part of a larger 273 microlitres (μl) cluster of contiguous significant voxels. The cluster size
was determined through 1,000 Monte Carlo simulations and, when combined with the
voxelwise statistical threshold, resulted in a 5% probability of a cluster surviving due
to chance. To compare activations between the CON and AR groups, the thresholded
activation maps were combined across groups. The combined maps included the
significant voxels of either group. This process was performed independently for the
STOPS and ERRORS trials. The mean activation for clusters of activation in the
combined maps was calculated for each participant and these functionally-defined,
region-of-interest means were used for between-group independent *t*-tests.

2.3.6 Voxel-based morphometry (VBM) analysis

VBM is a whole-brain analysis technique that is used for the characterisation
of regional cerebral volumes and the quantification of tissue concentration differences
in anatomical MR images (Ashburner & Friston, 2000). Optimised VBM (Good, et al.,
2001) was performed using FSL 4.0 VBM software (Smith, et al., 2004). First the
participant’s structural T1 images were brain extracted. Then segmentation was
performed in original space using FAST. The resultant GM partial volume images
were then aligned to the MNI152 standard space using the affine registration tool
FLIRT. The resulting images were then averaged to create a study-specific template
to which the native GM images were nonlinearly re-registered. The registered partial
volume images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 4.2 mm FWHM.

Voxelwise differences in GM between CON and AR groups were tested with the non-parametric Mann-Whitney test. These data underwent the same thresholding technique employed in the functional analysis. Significant voxels passed a voxelwise statistical threshold ($t(23)=2.81, p<.005$) and were required to be part of a larger 320 $\mu$l cluster of significant voxels. The results presented are significant at a $p<.05$, corrected for multiple comparisons.

### 2.3.7 Diffusion tensor imaging (DTI) analysis

DTI assesses WM connectivity in the brain by measuring the diffusion of water inside nerve fibre bundles. Fractional anisotropy (FA) quantifies the degree of non-random diffusion, provides a proxy for WM integrity. Statistical analysis of the FA data was carried out using tract-based spatial statistics (TBSS) (Smith, et al., 2006).

Pre-processing involved the reconstruction of the diffusion tensors to create an FA map, which was generated using DTIFit within the FMRIB Diffusion Toolbox. The data were normalised to the FMRIB58_FA map, a standardised template of 58 high-resolution FA images averaged from healthy participants. The tract-based analysis approach creates a skeleton of tracts that participants have in common. For each subject, a search is carried out along an invariant tract determined by the template and the voxel with the highest FA value is selected by comparing the FA value of the two nearest neighbours on each side in the direction perpendicular to the tract. No spatial blurring was required.
The CON and AR groups were compared using a Mann-Whitney statistical analysis. Significant voxels passed a voxelwise statistical threshold \( t(23)=2.81, p<.005 \) and were required to be part of a larger 10\( \mu \)l cluster for the FA analysis. (Note: Monte Carlo simulations revealed that 4\( \mu \)l was sufficient to avoid false positives at \( p<.05 \), corrected, but this criterion produced an excessively high number of group differences due, presumably, to the absence of spatial blurring of the DTI data.) The results presented are significant at a \( p<.05 \), corrected for multiple comparisons.

### 2.3.8 Imaging results and symptomatology analysis

A Pearson’s bivariate correlation within the AR and CON groups was conducted to examine the relationship between the subject’s current CGAS score of global functioning and percentage of STOPS, the fMRI activation levels in those regions shown to differ between groups for STOPS and ERRORS, the GM VBM mean scores and the DTI FA mean scores in those regions that differed between groups. Note that the ROIs used in the correlation analysis were exploratory and purely data-driven, therefore these results do not afford for an independent examination of the data.

### 2.4 Results

#### 2.4.1 Demographics

The sample consisted of 25 children: 14 children with no history of psychotic symptoms (CON), and 11 children who had experienced a “definite” psychotic symptom (AR). Participants ranged in age from 11-13 years of age. All participants were Caucasian and were attending their seventh or eighth year in mainstream primary schools. Across both participant groups the duration of time between the
clinical interview and neuroimaging session was between one and three months. There were no group differences observed between the length of time between their clinical interview the MRI scanning session. The reason for the delay between the interview and the scanning session was due to a technical issue with the MRI scanner.

Results from two-tailed \( t \)-tests revealed no significant group differences for age at date of scanning (in weeks) \([\text{CON}: 651\pm22, \text{AR}: 634\pm33; t(23)=1.42, p=.17]\), handedness \([\text{Edinburgh handedness inventory score: CON}: 62\pm30, \text{AR}: 45\pm46; t(23)=1.12, p=.28]\) and basic academic skills \([\text{WRAT-4 reading total score (out of 60)\: CON}: 54\pm15, \text{AR}: 50\pm5; t(23)=.92, p=.37]\). Chi-square tests revealed no differences between the groups for gender \([\text{CON}: 79\% \text{ female} (n=11), \text{AR}: 64\% \text{ female} (n=7); \chi^2=6.8, p=.41]\), family history of psychosis \([\text{CON}: 14\% (n=2), \text{AR}: 27\% (n=3); \chi^2=1.46, p=.48]\) or obstetric complications \([\text{CON}: 43\% (n=6), \text{AR}: 45\% (n=5); \chi^2=.02, p=.89]\). There was a small difference between the groups for years of education which reached statistical significance \([\text{CON}: 5.9\pm.4, \text{AR}: 5.4\pm.5; \chi^2=6.51, p=.01]\). There was a significant difference between groups on the CGAS, which is a measure of the children’s current global functioning (out of 100) \([\text{CON}: 88\pm9, \text{AR}: 78\pm11; t(22)=2.42, p=.02]\).

2.4.2 fMRI task performance

The AR children exhibited poorer inhibitory performance during the GO/NOGO task relative to the CON group, but this difference was not significant \([\text{CON}: 41\% \text{ vs. AR}: 29\% \text{ correct;} t(23)=1.83, p=.08]\) (Table 2.1). There were no significant differences in reaction times (RT) between groups for GO trials \([\text{CON}: 684\pm47ms, \text{AR}: 670\pm90ms; t(23)=.53, p=.6]\) or ERRORS \([\text{CON}: 632\pm34ms, \text{AR}: 639\pm72ms; t(23)=-.33, p=.74]\).
Table 2.1
ABD study behavioural performance during the GO/NOGO task.

<table>
<thead>
<tr>
<th>Group</th>
<th>STOPS</th>
<th>ERRORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (n= 14)</td>
<td>21±8</td>
<td>29±8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>21±8</td>
<td>29±8</td>
</tr>
<tr>
<td>Range: Min-Max</td>
<td>7-39</td>
<td>11-43</td>
</tr>
<tr>
<td>AR (n= 11)</td>
<td>15±8</td>
<td>35±8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>15±8</td>
<td>35±8</td>
</tr>
<tr>
<td>Range: Min-Max</td>
<td>4-31</td>
<td>19-46</td>
</tr>
</tbody>
</table>

2.4.3 fMRI activation

The activation patterns of the groups differed with greater right hemispheric activation for STOPS and ERRORS in CON and less extensive and more left hemispheric activation in the AR group (Figure 2.3). During the STOPS trials the CON group had significantly greater activation in six regions compared to the AR group (Table 2.2 and Figure 2.4). The areas were predominantly right lateralised and included the right inferior frontal gyrus (IFG), right precuneus, right lentiform nucleus, right middle temporal gyrus, left middle temporal gyrus, and left claustrum. There were four brain regions with greater activity in the AR group and these were left lateralised and included the left medial frontal gyrus/anterior cingulate (ACC) (BA32), left superior frontal gyrus, left ACC (BA24), and left precentral gyrus.

Reduced activity in the AR group was found for ERRORS in five regions, all right lateralised, including the ACC (BA32), posterior cingulate (BA23), right insula, middle frontal gyrus, and superior frontal gyrus (Table 2.3 and Figure 2.5). Conversely, the AR group showed increased activity compared to the CON group during ERRORS, all left lateralised, within the medial frontal gyrus, left superior frontal gyrus, and left middle occipital gyrus.
### 2.4.4 VBM

The AR group had significantly larger GM volumes in four regions: left middle temporal gyrus at Talaraich coordinates (LPl: -60, -48, 0), left superior temporal gyrus (-54, 16, -18), left angular gyrus (-50, -74, 36), and right orbitofrontal gyrus (18, 36, -26) than compared to the CON group (Figure 2.6A-D). The CON group had significantly larger GM volume in the left inferior temporal gyrus (-68, -28, -14) (Figure 2.6E).

### 2.4.5 DTI

Two participants were not included in the DTI analysis (one CON subject due to excessive head movement and one AR subject who did not complete the entire MRI session due to fatigue). There were three regions with significantly decreased FA values in the AR group relative to the CON group (Figure 2.7). The relevant fibre tracts included: inferior fronto-occipital fasciculus, within the right lingual gyrus centred at Talaraich coordinates (LPI: 4, -91, 0); cingulum bundle, within the left parahippocampal gyrus (-27, -20, -30); and the inferior longitudinal fasciculus, within the left STG (-37, -34, -6).

An exploratory FA analysis at a lower volume size of four µl explored whether regions linking the frontal and temporal lobes showed WM deficits. Two regions, most likely falling along the UF, showed evidence of group differences in fronto-temporal connectivity: one fell within the left inferior temporal lobe (-57, -5, -32) (CON>AR) and one in the left superior temporal lobe (-34, 10, -39) (AR>CON). Interestingly, these regions lie near those showing volumetric differences in GM. (Note: in the absence of higher-resolution DTI data which would enable tractography, the localisation of these effects to the UF cannot be confirmed).
Figure 2.3. Activation during STOPS and ERROR trials. (A) CON activity during STOPS \([t(13)=3.37, p<.005]\) (blue). (B) AR activity during STOPS \([t(10)=3.58, p<.005]\) (red). (C) CON group activity during ERRORS (blue). (D) AR group activity during ERRORS (red).
Table 2.2
Activated regions for STOPS during the GO/NOGO task.
Brain regions that revealed activity in both the AR and CON groups during STOPS ($p<.05$, corrected). Post-hoc t-tests ($p<.05$) revealed specific regions of activation differences between groups and are represented in the far right column.

<table>
<thead>
<tr>
<th>Region</th>
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<th>y</th>
<th>z</th>
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*p<.05; **p<.01; ***p<.005
Table 2.3
Activated regions for ERRORS during the GO/NOGO task.
Brain regions theta revealed activity in both the AR and CON groups during ERRORS (p<.05, corrected). Post-hoc t-tests (p<.05) revealed regions of activation differences between groups and are represented in the far right column.

<table>
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*p<.05; **p<.01; ***p<.005
Figure 2.4. Group differences in STOP-related activity for CON>AR (blue) and AR>CON (red) \(p<0.05\), corrected.

Figure 2.5. Group differences in ERROR-related activity for CON>AR (blue) and AR>CON (red) \(p<0.05\), corrected.
Figure 2.6. VBM results. GM increases in AR>CON (red) within the (A) right orbitofrontal gyrus (B) left middle temporal gyrus (C) superior temporal gyrus (D) left angular gyrus ($p<.05$, corrected). GM increase in CON>AR (blue) within the (E) left inferior temporal gyrus ($p<.05$, corrected).
2.4.6 Imaging results and symptomatology

The correlational analysis conducted to examine the relationship between the AR and CON subject's current CGAS score of global functioning. AR participants revealed a significant correlation between their CGAS score and percentage of STOPS ($r=-.65, p=.04$) and left ACC ($r=.67, p=.03$). CON participants did not reveal any significant correlations with CGAS score and behavioural performance, fMRI, VBM or DTI measures.
2.5 Discussion

Findings from the current investigation into the neurobiological correlates of risk in children experiencing subclinical psychotic symptoms revealed specific regional deficits which exist within a larger network of dysfunctional neuronal connectivity. The PFC has shown to be strongly functionally interconnected to the temporal lobe (Fuster, Bauer, & Jervey, 1985). This network has shown reductions in functional activations in both medicated patients with schizophrenia (Catafau, et al., 1994; Friston, Liddle, Frith, Hirsch, & Frackowiak, 1992) and neuroleptic-naïve patients (Andreasen, et al., 1997). The disconnection syndrome is observed in the AR group, which is shown in the reduced activity of the right PFC and temporal cortices, during the response inhibition task. This phenomenon reveals a potential indicator of disrupted fronto-temporal activity that may be underlying the behavioural trend in poorer performance on the task. A reduction in WM integrity within the connective fabric of the cortex, derived from the FA analysis, may give rise to the inefficient and compensatory processing in the brain. This was evidenced with the reduction in FA observed within the left temporal lobe which could be a structural deficit underlying decreased temporal functioning and hyperactive left PFC functioning due to less efficient connectivity within a complex network of fibres connecting the temporal lobe to frontal and occipital cortices. A disruption of this connectivity would give rise to learning and memory impairments that are mediated by these interconnected cortical regions (Browning & Gaffan, 2008).

2.5.1 fMRI

This study aimed to identify brain regions related to vulnerability to psychosis. Our fMRI task revealed activations in expected DLPFC, midline, and subcortical regions during response inhibition (Garavan, Ross, Murphy, Roche, & Stein, 2002),
regions also reported to be impaired in schizophrenia (Kindermann, Karimi, Symonds, Brown, & Jeste 1997). The fMRI results revealed reduced right hemispheric activation in the AR group in combination with left lateralised activation. The AR group revealed reductions in bilateral temporal and right IFG activity coupled with what may be interpreted as compensatory hyperactivation within left lateralised prefrontal cortices when successful inhibitions were made. Interestingly, the AR group was showing hyperactivity during STOPS in the left anterior cingulate which may reflect a compensatory strategy to deal with inefficient right PFC functioning to keep up with the on-line processing demands of the task.

During ERRORS, the CON participants engaged anticipated frontal, cingulate, and insular error processing brain regions (Hester, Fassbender, & Garavan, 2004). Hypofrontality is among one of the most replicated fMRI findings in schizophrenia (Glahn, et al., 2005). Results from the Edinburgh High Risk Study (Marjoram, et al., 2006) revealed reduced activity within the right PFC amongst individuals at genetic-risk for psychosis who were currently experiencing psychotic symptoms, but not in those at-risk with no symptoms. The finding of frontal hemispheric differences within the AR group is consistent with findings among individuals with chronic schizophrenia who have shown reductions in right lateralised PFC during response inhibition (Kaladjian, et al., 2007).

The finding of decreased cingulate activity in the AR group during ERROR trials adds to the growing evidence of pathological changes in this region preceding the onset of psychosis (Borgwardt, et al., 2007, Douaud, et al., 2007; Fornito, et al., 2008; Pantelis, et al., 2005). Children in the CON group revealed increased activity in dorsal ACC during ERRORS relative to the AR group. Error-related activity has been localised in the dorsal ACC and also serves as a crucial brain region in conflict
monitoring and has been shown to be impaired in patients with schizophrenia (Carter, MacDonald, Ross, & Stenger, 2001). Conversely, the AR children revealed hyperactivity in the rostral ACC during STOPS. Activation in this brain region has been observed during the resting-state and is a component of the default-mode network (Gusnard, Akbudak, Shulman, & Raichle, 2001). The rostral ACC is also associated with task-induced deactivations during cognitive tasks and overseeing emotional processing (Bush, et al., 1998; Bush, Luu, & Posner, 2000). Therefore, the AR children’s inability to deactivate the rostral region of the ACC during the cognitive task coupled with their failure to bring the dorsal ACC on-line during conflict monitoring reveals impairment in these dissociable regions of the ACC which are observable in the risk stage.

The positive correlation in the AR group between better global functioning scores and increased activity in the left ACC may reveal a brain region which is related to the general behavioural functioning in children. This suggests that children with better general functioning (i.e. interactions and behaviours at home, school, and with peers) have an increased ability to recruit the ACC, which is required from the participant to successfully complete the task. Fusar-Poli et al. (2009a) discovered that in a sample of ARMS subject’s increased left PFC was related to poorer accuracy on a verbal fluency task and reduced global functioning scores. However, at a one-year follow-up left PFC functioning had normalised in the ARMS group to similar levels of controls, which was positively correlated with an improvement of severity of psychotic-like symptoms. Therefore, a relationship seems to exist between left hemispheric frontal activity and a reduction in general functioning.

The hypoactivity within the right hemisphere, specifically within frontal and temporal regions, and compensatory left lateralised bias in the AR children contrasts
with the typical right hemisphere dominance attributed to response inhibition (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Chambers, et al., 2007; Garavan, Ross, & Stein, 1999; Konishi, Nakajima, Uchida, Sekihara, & Miyashita, 1998). An EEG study of auditory responses (Gruzelier & Manchanda, 1982) found a relationship between positive psychotic symptoms and a left>right asymmetry during the orienting response in schizophrenia patients. A correlation study found a relationship between greater scores on the positive schizotypy rating scale in control participants and increased left lateralised asymmetry during an auditory task (Mason, Claridge, & Clark, 1997). In the current AR sample these children exhibited a reduced bias in right>left asymmetry revealed by lower positive scores on the Edinburgh Handedness Inventory, which may have contributed to the less right-biased activity in this group. Combined, these results indicate a relationship between functional lateralisation and the appearance of positive psychotic symptoms.

2.5.2 VBM

I found reduced GM in the left inferior temporal lobe in the AR group. This echoes findings from the Edinburgh High Risk Study (Job, Whalley, Johnstone, & Lawrie, 2005) which found that in individuals at high genetic-risk for developing psychosis, who subsequently went on to develop schizophrenia and had reductions in the inferior temporal gyrus. An adolescent-onset schizophrenia study (Douaud, et al., 2007) observed a GM reduction in inferior temporal gyrus (BA 20) relative to adolescent controls. Additionally, a study examining GM volume within first-episode schizophrenic and affective psychosis patients revealed both groups had a reduction in inferior temporal lobe GM volumes, therefore revealing an underlying structural commonality within individuals with psychotic symptoms (Kuroki, et al., 2006b). In contrast to this area of GM reduction, the present results also identified increased GM
within the superior and middle temporal gyri of the AR group. A study of children with childhood-onset schizophrenia (Jacobsen, et al., 1996) reported a similar effect of increased temporal lobe volumes in patients, specifically in the superior temporal gyrus. However, most at-risk studies have examined an older cohort aged 18 and above (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005; Job, Whalley, Johnstone, & Lawrie, 2005) therefore, changes within the temporal cortex, such as synaptic pruning, which may still be ongoing at the time of testing and the finding of increased GM may indicated a delay in neuronal pruning, since the STG and PFC are the last areas of the cortex to develop (Thompson, et al., 2001).

Temporal lobe abnormalities have been consistently associated with positive symptoms in chronic schizophrenia (Kim, et al., 2003a; Shenton, et al., 1992; Turetsky, et al., 1995). A convergence between morphometry and function is highlighted in our finding of left lateralised functional activation in the AR group during the GO/NOGO task and increased GM volume. These observations suggest that the left temporal lobe may provide a particularly sensitive marker of early psychotic symptoms which may be valuable in identifying those at-risk of subsequently developing psychosis.

2.5.3 DTI

The DTI analyses revealed reductions in WM integrity in the AR children within the right lingual gyrus, left parahippocampus, and left STG. Impairments in the functioning of the fronto-occipital early visual processing region may be related to reductions in P100 amplitude that have been observed in schizophrenia patients and their first-degree relatives (Yeap, et al., 2006) and have been associated with polymorphisms of the dysbindin gene (DTNBPl) (Donohoe, et al., 2008). The inferior fronto-occipital fasciculus spans from the frontal lobe through the temporal
lobe continuing to the occipital lobe (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005). According to the disconnection syndrome an insult to the association fibre connecting the frontal and occipital lobes would cause impairments in the ability to connect the visual cortex to the language centres in the brain. DTI research probing the disconnection hypothesis has revealed that WM FA reductions along tracts in the PFC provide a structural basis for age-related cognitive decline (O’Sullivan, et al., 2001). A disruption of WM integrity along the inferior fronto-occipital fasciculus, which passes through the middle temporal lobe, could be an underlying cause of the functional reductions in normal response inhibition patterns observed in the AR children.

FA reductions in the AR group were also observed in the left parahippocampus along the cingulum. The cingulum bundle tracts carry afferent connections from the cingulate gyrus to the frontal lobe connecting the precuneus, hippocampus and parahippocampus (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005) and may be an important element of coordinated inter-regional cortical functioning. An aberration within parahippocampal connectivity could lead to memory difficulties, since this region is the first to receive sensory information before passing through the entorhinal cortex. Resting-state functional connectivity analysis reveals correlations in activation patterns between the precuneus/posterior cingulate cortex and medial frontal cortex of the default-mode network (DMN) (van den Heuvel, Mandl, Luigjes, & Hulshoff Pol, 2008), which both lie on the cingulum tract. These synchronous activation patterns between these defaults regions, which were shown to be abnormal during functional imaging in the AR group, could be caused by a deficiency in micro-structural organisation along the cingulum.
Finally, the reduction in FA along the inferior longitudinal fasciculus, connecting the occipital and temporal lobes, falls within the left STG, which has been suggested to be a locus of hallucinations among those with schizophrenia (Pearlson, 1997). Reduced axonal integrity, and subsequent signalling between the temporal and occipital lobes, gives rise to the hypothesis that an aberration within this region could contribute to the appearance of the positive psychotic symptoms.

2.6 Conclusions

The present study suggests that the vulnerability to psychosis can be examined within the context of development. This study reveals that the developing brains of the at-risk children, presenting psychotic-like symptoms, and aged 11 to 12 years old, show mainly right lateralised prefrontal-temporal dysfunctions, focal GM volume increases and WM micro-structural reductions. These multi-modal findings provide a broad neurobiological characterization of individuals at symptomatic risk for developing psychosis. The results of the fMRI, VBM and DTI analyses support the hypothesis of a prefronto-temporal structural disconnectivity. Future corroboration could come from higher-resolution DTI data enabling delineation of the WM tracts, particularly the UF tract connecting frontal and temporal brain regions. Due to the difficulty involved in the recruitment of this unique population the small sample size of this study was relatively small and may have not allowed for more subtle deficits in this developing cohort to survive the stringent statistical thresholds employed. Additionally, we were not afforded the ability to have within-group matching for gender. This resulted in an over-representation in the sample of female participants. Although the study groups (AR and CON) were matched for gender and lifetime risk for both sexes is around 1%, the development of schizophrenia has shown differences in onset with respect to males and females. For example, men typically develop
schizophrenia on average 4-6 years earlier than females (Häfner & Nowotny, 1995). In future research matching the gender within-subject groups will be an important selection criterion to adhere to, which will avoid the known clinical and maturational differences between males and females.

This AR group should not be conceptualised as a “prodromal” cohort, but rather a group of non-treatment seeking children who exhibit a risk phenotype based on the presence of subclinical psychotic symptoms. While there is an increased risk for clinical psychosis among children reporting subclinical psychotic symptoms, the majority of individuals (~35-84%) experiencing psychotic symptoms do not develop psychosis (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005; Poulton, et al., 2000; van Os, Linscott, Myin-Germeyns, Delespaup, & Krabbendam, 2009). Consequently, findings within this at-risk group add to our greater understanding of how brain variation at an early age, and perhaps at an earlier age then previously assumed to be detectable, can relate to the development of psychotic symptoms.
3. Chapter Three
Chronic schizophrenia patients and children at-risk: A multi-modal cross-sectional study of structural and functional neural correlates of psychosis
3.1 Abstract

Studying children experiencing psychotic symptoms provides a unique opportunity to examine vulnerability to psychosis within the context of development. Using neuroimaging techniques, this study investigated cognitive control functions, brain morphometry, and white matter integrity in an at-risk cohort of children, from the Adolescent Brain Development study (ABD study) (Jacobson, et al., 2010; see Chapter Two), and patients with chronic schizophrenia. A between-subjects assessment of brain function and structure was conducted on 11 children at symptomatic risk (AR) for developing psychosis and 14 healthy control children (Con-C) and separately for 17 medicated schizophrenia patients (Sz) and 16 matched healthy adult controls (Con-A) in order to determine the patterns of functional and structural abnormalities found in both psychosis groups, independent of the stage of the disease data from the two psychosis (PSY) groups and two control (CON) groups were pooled in a combined group analysis. I investigated: (1) functional measures of response inhibition, (2) VBM analyses of grey matter (GM), and (3) DTI exploring fractional anisotropy (FA) to assess white matter (WM) integrity. Functional magnetic resonance imaging (fMRI) results revealed reduced activity in the PSY group within the right dorsal lateral prefrontal cortex, right ventral lateral prefrontal cortex, left middle temporal gyrus and claustrum \((p<.05, \text{corrected})\). The VBM analysis revealed a GM reduction in the PSY group within the right insular cortex \((p<.05, \text{corrected})\). The DTI analysis identified WM FA reductions in the PSY group in bilateral parahippocampus, posterior cingulate gyrus, right inferior parietal lobule, right postcentral gyrus, ventral striatum, and fornix \((p<.05, \text{corrected})\) and increases in FA in the dorsal striatum, pons, and right ventral anterior cingulate gyrus. The functional and structural abnormalities found in common in the PSY group may reveal early
biomarkers related not only to the vulnerability for developing psychosis but persistent in the underlying pathology present in those who have developed the disease.
3.2 Introduction

3.2.1 Prefrontal functioning and cognitive control in schizophrenia

The research of Bleuler (1911) and Kraepelin (1919) first posited that schizophrenia was a disease originating in the brain, with cognitive disturbances being the central feature of the illness. Neuroimaging research over the last three decades to elucidate the complex neural basis of schizophrenia has provided an emerging picture of the underlying neuropathology of the illness. The most consistent neuroimaging finding in schizophrenia has been functional abnormalities within the prefrontal cortex (PFC) (see meta-analysis in Hill, et al., 2004). This region has been conceptualised as the central orchestrator in higher-order tasks of executive function and is dysfunctional in schizophrenia (Weinberger, Berman, & Zec, 1986). The PFC is thought to be related to the negative and disorganised symptoms expressed in the illness (Goldman-Rakic & Selemon, 1997). A recent meta-analysis of 41 fMRI studies examining executive functioning in schizophrenia found that the most common hypoactivity during cognitive processing to lie within the dorsal lateral prefrontal cortex (DLPFC) (see review in Minzenberg, Laird, Thelen, Carter, & Glahn, 2009). Neuroimaging research has identified the DLPFC as a critical locus of top-down executive processing in the brain (Fassbender, et al., 2009; MacDonald, Cohen, Stenger, & Carter, 2000; Smith, Jonides, Marhuetz, & Koepppe, 1998). Evidence of hypoactivity in the DLPFC in schizophrenia patients have been observed during executive tasks of working memory (Glahn, et al., 2005) response inhibition (Rubia, et al., 2001), and context processing in un-medicated first-episode patients (Barch, et al., 2001). Individuals at an elevated genetic risk for the illness have also shown functional abnormalities in the DLPFC for response inhibition (Vink, Ramsey, Raemaekers, & Kahn, 2006), language processing (Sommer, Ramsey, Mandl, van Oel,
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Cross-sectional study of the risk for psychosis

& Kahn, 2004), and working memory (Brahmbhatt, Haut, Csernansky, & Barch, 2006; Callicott, et al., 2003; Karlsgodt, et al., 2007; Keshavan, et al., 2002; Thermenos, et al., 2004). However, instances of hyperactivity in the DLPFC in schizophrenia patients have also been found. Weinberger and McClure (2002) have attributed the hyperactivity to inefficient and less automatic processing within the PFC, although the participant may be performing well on a task, which results in the extraneous neural activity. Abi-Dargham et al. (2002) discovered that schizophrenia patients have increased dopamine (DA) D₂ receptors in the DLPFC, which was strongly correlated with poorer working memory performance. Therefore, the increased DA uptake might represent a compensatory, however inefficient process which is secondary to a more sustained deficiency in PFC DA functioning related to abnormal maturational processes which have been found in patients with schizophrenia (Weinberger, 1987).

An examination from post-mortem studies seeking to identify a structural causal mechanism for DLPFC dysfunction in schizophrenia found that patients had greater neuronal density in the DLPFC than controls (Selemon, Rajkowska, & Goldman-Rakic, 1998). They interpreted the observed elevation in neuronal density to be a result of reductions in interneuronal neuropil, which may be one underlying anatomical substrate for PFC dysfunctions in schizophrenia (Selemon, Rajkowska, & Goldman-Rakic, 1998).

Another region within the PFC that has consistently shown dysfunctions in schizophrenia patients is the inferior frontal gyrus (IFG) (BA 47), which is in the ventral lateral prefrontal cortex (VLPFC) (Minzenberg, Laird, Thelen, Carter, & Glahn, 2009). Neuroimaging studies have shown the right VLPFC is engaged during the set-shifting Wisconsin Card Sorting Task (WCST) (Berman, et al., 1995; Buchsbaum, Greer, Chang, & Berman, 2005; Konishi, et al., 1998), inhibitory control
processes (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Garavan, Ross, & Stein, 1999; Konishi, Nakajima, Uchida, Sekihara, & Miyashita, 1998), and set-shifting (Brass, et al., 2003; Dreher & Berman, 2002). A deficit in the IFG can result in the inability to inhibit irrelevant information. The IFG is one of the most well-connected regions in the PFC (Miller & Cohen, 2001), therefore a dysfunction in this region has the potential for a cascading effect of inefficient connectivity on the rest of the cortex. One particularly important link from the IFG is to the subthalamic nuclei, which has been shown to be important for motor inhibition (Aron & Poldrack, 2006).

A cross-sectional fMRI study of prodromal, first-episode and chronic schizophrenia patients which used a visual oddball task found dysfunctions within the VLPFC in all three patient groups (Morey, et al., 2005). An investigation of sustained attention in neuroleptic naïve patients found reduced IFG activity (Ojeda, et al., 2002). These studies reveal a decline in VLPFC functioning before the onset of the illness in regions important to conducting high-order cognitive processes which impact the expression of lower level processes, such as motor control. The identification of such a highly connected vulnerability biomarker has the potential to explain many of the underlying dysfunctions observed not only in those with the illness, but also in the early signs detectable in those at-risk.

Schizophrenia patients often find it difficult to filter out or inhibit irrelevant material in their environment (McGhie & Chapman, 1961). This filtering impairment can be quantified and assessed during cognitive inhibition tasks. One such task that has been suggested to elicit impairment in the processing of inhibiting irrelevant or inappropriate information or actions in schizophrenia is response inhibition. Response inhibition refers to the suppression of actions, responses, stimuli, thoughts, memories, or learned ways of performing a task that are inappropriate in a given behavioural
context or that are unwanted because they interfere with the completion of a motor or cognitive task (Fassbender, et al., 2004). The ability to inhibit inappropriate mental states, such as distracting endogenous and exogenous stimuli or inappropriate motor responses, is essential in executing goal-directed behaviours. The VLPFC and DLPFC regions have been commonly found to be the central regions activated during response inhibition (Blasi, et al., 2006). A meta-analysis of GO/NOGO motor response inhibition tasks, which required the participant to suppress a pre-potent response revealed prominent activation patterns in the right frontal cortex during successful inhibitions (STOPS) (Buchsbaum, et al., 2005). A separate meta-analysis of the “XY” GO/NOGO task, which is the task used in the current study, found right DLPFC and VLPFC activations for correct inhibitions (Garavan, Hester, Murphy, Fassbender, & Kelly, 2006). Fassbender et al. (2004) examined component processes during the Sustained Attention to Response Task (SART) and found that correct inhibitions were associated with activation in the right IFG and task-set maintenance was associated with the left DLPFC. Neuroimaging studies examining various response inhibition tasks in schizophrenia patients have indicated a deficit in the PFC during Stroop tasks (Barch, Carter, Hachten, Usher, & Cohen, 1999; Henik, et al., 2002), context-processing tasks (Javitt, Shelley, Silipo, & Lieberman, 2000), and GO/NOGO tasks (Ford, et al., 2004; Kaladjian, et al., 2007; Kiehl, Smith, Hare, & Liddle, 2000; Rubia, et al., 2001; Weisbrod, Kiefer, Marzinzik, & Spitzer, 2000).

3.2.2 Inefficiency hypothesis of schizophrenia

Hypofrontality has been attributed to participants performing poorly on a task, in which the demand of the task exceeds the participant’s abilities, and is evidenced by reduced activity within the DLPFC in schizophrenia patients (Callicott, et al., 2003b; Minzenberg, Laird, Thelen, Carter, & Glahn, 2009; Weinberger & McClure,
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2002). However, hyperfrontality has also been observed in schizophrenia patients and is thought to be a result of inefficient and less automatic processing within the PFC when the participant is performing well on a task and recruits extraneous neural activity (Weinberger & McClure, 2002). A possible explanation for this phenomenon is the inefficiency hypothesis, which states that when there is impairment in the region that is expected to be engaged for the task (i.e. the right IFG during response inhibition tasks or DLPFC during context processing), the patients reveal hyperactivations in this region to cope with their perceived difficulty of the task (Callicott, et al., 2003a). Ramsey et al. (2002) have suggested that the inefficiency of neural communication observed in schizophrenia patients results in excessive recruitment of neural systems to perform at comparable levels to controls during cognitive tasks. In a study of working memory functioning during an n-back task in first-episode patients, researchers found that during the 0-back condition first-episode patients activated the same extensive network of activity as the controls during the 2-back condition, but found overall reduced activity in the patients during the 2-back condition (Mendrek, et al., 2004). Thus, the normally undemanding 0-back task required patients to recruit available cerebral resources to near maximal capacity therefore rendered these regions exhausted for the demanding 2-back condition. Greater DLPFC activity has been found in unaffected siblings during the AX-CPT content-processing task during the low cognitive demanding condition (Delawalla, Csernansky, & Barch, 2008), whereas controls revealed hyperactivity in the DLPFC during the more difficult condition. Further evidence for the inefficiency hypothesis can be observed in the developmentally immature brains of school-age children who show more diffuse, less active, and widespread patterns of activation compared to

3.2.3 Neurodevelopmental model of schizophrenia

The neurodevelopmental theory of schizophrenia states that schizophrenia is the behavioural outcome of an aberration occurring in the pre- and perinatal neurodevelopmental process, which can also coincide with a combination of environmental and genetic factors (Singh, McDonald, Murphy, & O'Reilly, 2004). Schizophrenia as a neurodevelopmental disease (Weinberger, 1995) is evidenced by progressive GM and WM deficits beginning in the parietal lobe and eventually moving along an anterior path into the frontal lobe (Gogtay, et al., 2004; Thompson, et al., 2001; Vidal, et al., 2006). GM volume deficits in the parietal lobe have also been found in individuals at-risk for schizophrenia (Job, Whalley, Johnstone, & Lawrie, 2005) and around the time of transition from the prodromal state to psychosis (Pantelis, et al., 2005). This finding indicated that focal structural abnormalities are present before the onset of the illness and are likely to be linked to a disruption of normal brain developmental processes. Maturational delays in brain development have been found to be an indicator of susceptibility of an early onset of the illness in adolescents with schizophrenia (Hollis, 1995; Karp, et al., 2001). In normal childhood brain development the posterior regions (occipital and parietal) mature first, followed by temporal lobe refinement, and lastly frontal lobe maturation, which completes in the third decade of life (Casey, Giedd, & Thomas, 2000). Research examining sex differences in childhood and adolescent brain development have revealed that males have overall greater amounts of grey matter volume present during development, whereas females have increased grey matter in focal areas that are well-known to show reductions in schizophrenia patients within the mesial temporal lobes, caudate
and thalamus (Sowell, et al., 2002). These findings are important to consider when observing differences in maturation patterns between adolescent individuals at-risk, as observed effects may be driven by gender and not necessarily disease progression. Previous studies have revealed that the earliest maturing posterior brain regions were recruited in children’s brains during response inhibition and working memory tasks (Bunge, Dudukovic, Thomason, Vaidya, & Gabrieli, 2002; Ciesielski, Harris, & Cofer, 2004; Ciesielski, Lesnik, Savoy, Grant, & Ahlfors, 2006). Since the typical onset of schizophrenia normally coincides with the later stages of adolescence and more specifically the peak time of brain maturation in the frontal lobes, it becomes apparent that the onset of the illness may be related to aberrant development in this region of the cortex. Research exploring the relationship between adolescent white matter myelination increases in the PFC and more efficient neural processing during response inhibition has demonstrated the importance of maturation on optimal cognitive control (Stevens, Skudlarski, Pearlson, & Calhoun, 2009). Within this theoretical framework schizophrenia can be theorised to arise from cortical maturation delays which subsequently only reach an inferior level of maturation (DeLisi, 2008), which in turn gives rise to the inefficient cortical processing observed in schizophrenia patients.

In my previous study of response inhibition in children at-risk (Jacobson, et al., 2010; see Chapter Two) I found hypoactivity within the right hemisphere, specifically within frontal and temporal regions, and a compensatory left lateralised pattern of activity. This pattern of activity in the AR children contrasts with the typical right frontal hemispheric dominance found during response inhibition tasks (Aron, et al., 2003; Chambers, et al., 2007; Garavan, et al., 1999). However, our results are in line with current reports of at-risk mental state (ARMS) subject’s experiencing focal
reductions in right PFC (Broome, et al., 2009) and increased in left PFC functioning (Fusar-Poli, et al., 2009a). Additionally, Fusar-Poli et al. (2009a) found that at a one-year follow-up the ARMS subject’s whose left PFC functioning had normalised to similar activation levels as the controls had an improvement in the severity of their psychotic-like symptoms. Therefore, the left lateralised frontal activation, found during the response inhibition task could serve as a potential early biomarker of the risk for psychosis, which has clinical implications for the individual’s level of global functioning. Additionally, this left lateralised hyperfrontality could be a possible indicator of those who will not achieve optimal PFC development, since those with the established illness have reductions in PFC GM which are related to deficits in PFC functioning during cognitive tasks (Antonova, Sharma, Morris, & Kumari, 2004; Goldman-Rakic, 1995; Goldman-Rakic, 1999; Golman-Rakic & Selemon, 1997).

3.2.4 Disconnection theory of schizophrenia

In addition to functional abnormalities in the brain schizophrenia is also characterised by widespread grey and white matter deficits, which are associated with functional disturbances (see meta-analysis in Wright, et al., 2000). From such observations the hypothesis that schizophrenia is a disconnection syndrome has arisen and has become a prominent theory of the neuropathology of the illness. A basic premise of this theory is that within the structural cognitive networks of the brain communicate through an elaborate web of interconnecting WM fibres and when a focal disruption occurs the entire network will be adversely affected (Bullmore, Frangou, & Murray, 1997; Friston & Frith, 1995; Weinberger, Berman, Suddath, & Torrey, 1992). Friston (1998) posited that this functional disconnectivity is based on altered synaptic connection strength rather than on structural macro-anatomical changes. The disconnection hypothesis has received some empirical support from
functional brain imaging studies and points to potential mechanisms at the molecular level; however, there is a lack of consensus on which brain networks are the site of the primary abnormality. A possible locus of cognitive control has been suggested in basic neuroscience and lesion studies, to lie within the DLPFC which is an area integral to coordinating activity for goal-related behaviours (Desimone, 1996; Tomita, Ohbayashi, Nakahara, Hasegawa, & Miyashita, 1999). Disturbed connections between the PFC and temporal lobes and their subsequent integration with areas in the neocortex have been the most common findings (Friston & Frith, 1995; Lipska, 2004; Sigmundsson, et al., 2001; Weinberger & McClure, 2002; Weiss & Heckers, 2001). However, Miller and Cohen (2001) have proposed a model that posits that the DLPFC biases activity in the posterior cortex, which is necessary for controlled performance.

The parietal lobes have been less emphasised in schizophrenia research, but there is emerging evidence that fronto-parietal integration may be abnormal (Hugdahl, et al., 2004; Kim, et al., 2003b; Quintana, et al., 2003). Research in task-related functional connectivity has elucidated that a disruption between PFC and parietal communication may be at play. Yoon et al. (2008) conducted a functional connectivity study of activity during the AX-CPT. They found greater connectivity between the DLPFC and right inferior parietal lobule and left premotor cortex, however schizophrenia patients did not show any regions with enhanced functional connectivity to the DLPFC. Other studies have reported a specific impairment in functional coupling between the PFC and hippocampal regions (Heckers, et al., 1999; Meyer-Lindenberg, et al., 2005; Weinberger, Berman, & Torrey, 1992; Zhou, 2008). Recently, Benetti et al. (2009) explored effective connectivity during a working memory task in first-episode, ARMS, and healthy control subjects. They set out to assess bi-directional connectivity between the PFC and hippocampus, particularly
between the IFG which has been reported to have prominent projects to the posterior subdivision of the hippocampus and is known to be associated with the “disrupted in schizophrenia one” (DISC1) gene (Roberts, 2007). They examined the phase of correct recognitions to previously presented stimuli and found reduced effective connectivity from the right posterior hippocampus to the right IFG in the first-episode and ARMS subjects compared to controls. Findings of decreased intrinsic connectivity in subject’s at high-risk is consistent with reports of abnormal PFC, posterior, and also subcortical brain region functional connectivity (Whalley, et al., 2005). Other recent reports have associated the ARMS with focal reductions in right PFC (Broome, et al., 2009), increased in left PFC (Fusar-Poli, et al., 2009a), and reductions medial temporal cortices (Borgwardt, et al., 2007; Morey, et al., 2005; Pantelis, Yücel, Wood, McGorry, & Velakoulis, 2003). Disruptions in prefrontal-hippocampal connectivity are rapidly becoming a compelling vulnerability marker in individuals who are at-risk.

3.2.5 White matter disruptions in schizophrenia

A relatively new MRI technique to examine structural connectivity and WM abnormalities is DTI. FA is a frequently used measure of the degree of diffusion anisotropy, or directional dependency, found along a WM fibre within a given voxel in the brain. FA is derived from DTI data to infer fibre organisation and directional coherence (Beaulieu, 2002). Thus, a finding of decreased FA would indicate a WM abnormality pertaining to axonal disorganisation.

One WM tract of interest showing reduced FA in first-episode of schizophrenia patients is the uncinate fasciculus (UF) (Price, et al., 2008). This fibre bundle connects the VLPFC to the temporal pole via the amygdala (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005). An FA reduction along this fibre has been
associated with reduced executive functioning in schizophrenia patients (Kubicki, et al., 2002). Lower FA along the uncinate fasciculus has also been shown to correlate with an increase in negative symptoms in individuals with recent onset schizophrenia (Szeszko, et al., 2008).

The cingulate gyrus, located along the cingulum fibre bundle, is another region of interest derived from empirical research in schizophrenia. Neuroimaging research has revealed functional deficits in the cingulate during response conflict tasks which were coupled with impaired affective responses (Laurens, Ngan, Bates, Kiehl, & Liddle, 2003; Polli, et al., 2008). This bundle connects the PFC, cingulate gyrus, medial temporal lobe, including the hippocampus and parahippocampus, and precuneus (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005). WM of the cingulum has previously revealed FA reductions in schizophrenia patients within the left frontal WM in a large meta-analysis of FA studies, n=407 schizophrenia patients and n=383 controls (Ellison-Wright & Bullmore, 2009). Other studies have revealed reductions in schizophrenia patients along the cingulum bundle falling within the PFC (Weiss & Heckers, 2001), cingulate gyrus (Fujiwara, et al., 2007; Kubicki, et al., 2003), and also in the hippocampus in children and adolescents with schizophrenia (White, et al., 2007). A combined study of DTI and fMRI assessed working memory functioning in schizophrenia patients and revealed hypoactivity in the PFC, superior parietal lobe, and occipital lobe and FA reductions in the right parahippocampal gyrus and right frontal lobe (Schlösser, et al., 2006). This study suggests that FA reductions falling along the cingulum are associated with reduced fronto-parietal-occipital functioning.

A final connectivity pathway which has shown reductions in FA in schizophrenia is the arcuate fasciculus (Burns, et al., 2003; Kubicki, et al., 2005). The arcuate fasciculus is a tract connecting the regions of language and auditory
perception, Broca and Wernicke’s areas and is believed to play a role in integration cognition and emotion (Barbas, Ghashghaei, Dombrowski, & Rempel-Clower, 1999). Adolescent-onset schizophrenia patients have been found to show reduced FA along the arcuate fasciculus and also in the corticospinal tract (Douaud, et al., 2007).

Previous reports of FA in individuals at-risk have used a genetic-risk approach studying unaffected relatives of schizophrenia patients. These studies have revealed that relatives show WM deficits in left PFC and the hippocampus (Hao, et al., 2009), left parahippocampus (DeLisi, et al., 2006), anterior limb of the internal capsule (Muñoz Maniega, et al., 2008), along the superior longitudinal fasciculus (SLF) (Karlsgodt, Niendam, Bearden, & Cannon, 2009), left posterior cingulate and right parietal lobe (Hoptman, et al., 2008). Specific FA reductions in the left ACC have been found in patients but not in unaffected relatives (Hao, et al., 2009). Therefore, left fronto-hippocampal, fronto-temporal, and parieto-cingulate WM deficits may be related to a genetically-mediated indicator of a higher vulnerability to develop schizophrenia to higher genetically-mediated to develop schizophrenia, however WM disruptions in the left ACC may be specific to the presence of the disease.

To date there have not been any studies that have investigated FA in individuals who are experiencing psychotic-like symptoms. However, one study which examined probabilistic fibre tracking in at-risk individuals who were experiencing psychotic-like symptoms did not find any differences in WM tractography between either at-risk, first-episode or controls (Peters, et al., 2008). However, this study only examined male participants, which is not a representative sample of schizophrenia patients. Also the lack of WM difference between the schizophrenia patients and controls raises questions about their methodology, as DTI WM abnormalities are a well replicated finding in schizophrenia research (Ellison-
Wright & Bullmore, 2009). An investigation of whole-brain DTI examining FA in a young at-risk population could aid in the explanation of the aberrant behavioural and functional performance during cognitive control tasks. FA analyses, coupled with fMRI findings, have the potential to determine the integrity of structural connectivity pathways between brain regions that have shown reductions in individuals with schizophrenia.

3.2.6 The symptomatic at-risk approach to studying schizophrenia

More recent approaches to understanding the functional and structural aetiology of schizophrenia have used an ultra-high risk (UHR) approach, which involves identifying individuals in late adolescence who are seeking treatment for prodromal symptoms of psychosis (Cannon, et al., 2008; McGorry, et al., 2002). Individuals identified in the UHR stage have been found to have a 10-40% risk of developing a psychotic disorder in the next 24 months (Miller, et al., 2003; Yung, et al., 2003). An alternative high risk approach involves studying children at symptomatic risk of psychosis, that is, children who report subclinical psychotic symptoms. Longitudinal research has shown that children who report subclinical psychotic symptoms are at significantly increased risk, ranging from 16- to 65-fold increase, of developing a clinical psychotic disorder (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005; Poulton, et al., 2000; van Os, Linscott, Myin-Germeyns, Delespaup, & Krabbendam, 2009). Poulton and colleagues (2000) conducted a birth cohort study in New Zealand and found 11-year olds with self-reported psychotic symptoms were at a 16-fold increased risk for an adult psychotic illness. Kelleher and colleagues (2009) investigated this instance of self-reporting of psychotic-like symptoms by testing the validity of the self-report psychosis screening instrument, used in the aforementioned study and in our present study, which they have named the Adolescent Psychotic-Like
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Symptom Screener (APSS). The validation study of the APSS revealed that this quickly administered screening tool (administration time is less than five minutes) has a positive predictive value (PPV) of 71% and a negative predictive value (NPV) of 90% of detecting psychotic-like symptoms.

I conducted a cross-sectional design between children at-risk and adult schizophrenia patients to assess brain structure and function related to the established illness and the risk for developing schizophrenia. Two separate studies were conducted and included a chronic schizophrenia study (ChSz study) of schizophrenia patients and healthy adult controls, and the ABD study (Jacobson, et al., 2010; see Chapter Two), which included children who were experiencing subclinical psychotic symptoms and asymptomatic healthy control children. We applied a combined group analysis between the two studies conjoining the at-risk children and chronic patients, which will be referred to as the PSY group and, separately, the children and adult controls in the comparison group, referred to as the CON group. Conjunction analyses have been shown to be more accurate in assessing activation differences between groups, rather than simply a subjective comparison of the results of the separate studies (Price & Friston, 1997), although we used a slightly modified approach on the standard conjunction analysis (Nichols, Brett, Andersson, Wager, & Poline, 2005) of the fMRI data which I will refer to as a “combined group analysis.” The VBM and DTI follow the standard method of a conjunction analysis and will be referred to as so.

The aims set forth in this study were: (1) to use fMRI to examine functional activations during an inhibition task in at-risk children and schizophrenia patients; (2) to examine frontal and temporal functioning in relation to vulnerability stage to determine which regions show a difference; and (3) to assess brain structures in relation to the vulnerability for psychosis by examining GM volume, using VBM, and
structural WM integrity, using DTI, in the PSY group compared to the healthy CON group. We hypothesised that the children identified as at-risk from the ABD study would show a common, yet less pronounced, patterns of functional and structural dysfunction, than those diagnosed with the disease, particularly within hypothesised prefrontal, medial and middle temporal, and inferior parietal regions.

3.3 Materials and methods

3.3.1 Participants

Chronic schizophrenia study (ChSz study)

The study of patients with chronic schizophrenic was approved by St. Vincent’s Hospital, Fairview, St. James’s Hospital Dublin, Beaumont Hospital, and the Trinity College School of Psychology research ethics committees. Recruitment for chronic schizophrenia patients was co-ordinated through St. Vincent’s Hospital, Fairview, St. James Hospital Dublin, and Beaumont Hospital. They were recruited using a patient information leaflet which was handed out at outpatient clinics or during an appointment with their clinical psychologist or psychiatrist in the hospital (see Appendix Eight). Potential participants were encouraged to contact one of the studies’ researchers should they have any questions. The participants were then contacted by a researcher by telephone to enquire if they would like to participate in the imaging study. Following a complete description of the study, all 33 participants gave written consent to participate in the study (see Appendices Three and Nine). The sample consisted of 17 participants with schizophrenia (Sz) (Age range: 26-61) and 16 healthy adult controls (Con-A) (Age range: 25-58) who were matched on an individual paid basis to the patients (Table 3.1). All participants, except one Sz patient, were right-handed as measured by the Edinburgh Handedness Inventory. The Sz patients were assessed using the Positive and Negative Syndrome Scale (PANSS)
Kay, Fiszbein, & Opler, 1987) to rate positive and negative psychopathology. In the Sz group, 13 participants were diagnosed with paranoid schizophrenia and four had a diagnosis of schizoaffective disorder, as defined by the criteria detailed in the Diagnostic and Statistical Manual of Mental Disorders 4th Edition-Revised (DSM-IV-TR) (APA, 2000). The Sz participants were all outpatients who were stable on medication at the time of testing, and had previously participated in research studies at the hospital. The types of medication included atypical and typical antipsychotics and in some cases a combination of both. Prior to the start of the recruitment for the study, five patients with chronic schizophrenia were tested on the GO/NOGO response inhibition task. Data from this pilot testing revealed that patients had difficulty completing the task at the 1Hz speed of presentation; therefore a 1.5Hz speed of presentation was adopted. This increased response window produced higher accuracy rates in the patients and ease of understanding the task. Individuals who were not able to understand the task during practice testing before the scan were excluded from the fMRI portion of the scan (n=2 Sz and 1 Con-A). The ability to complete the test to accuracy which was comparable to control participants was necessary to ensure that the activations observed during the task were sufficient in number and related to the cognitive demands which were engaged during successful trials of the task.

Control participants were recruited from a participant subject pool from the School of Psychology at Trinity College Dublin. The Con-A group was composed of 16 healthy volunteers. Participants in this group did not have any history of any Axis I disorders and were matched to the Sz participants for gender, age, and education. Strict participant confidentiality was maintained throughout. Participants were assigned an anonymous code number following their first contact. The number was used throughout the experiment and was the only identifier on behavioural and MRI
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data. All results were available to participants on request. Participants were assured they could withdraw at any stage of the experiment.

The ChSz study was composed of a total of 33 participants who were scanned in the MRI. All participants were included in the VBM and DTI analyses, however, due to an inability to perform the functional task, two Sz patients and one Con-C participant were excluded from the fMRI analysis, resulting in an fMRI analysis of 15 Sz patients (Mean age: 44±12; range 26-61; 11 males; Mean years of education: 12±2.2) and 15 Con-A participants (Mean age: 44±11; range 25-58; 11 males; Mean years of education: 14±2.2).

Adolescent Brain Development study (ABD study)

The details of the identification of the participant sample for the study of Adolescent Brain Development study (ABD study) can be found in Chapter Two and additionally in Jacobson et al. (2010). Children who took part in the imaging study were recruited from schools in the greater north Dublin area. Individuals at-risk were initially identified through the APSS psychosis screening questionnaire (Kelleher, Harley, Murtagh, & Cannon, 2009) (see Chapter Two, Figure 2.2). Individuals who met \textit{a priori} screening score criteria were invited to undergo a clinical interview with a trained psychiatrist or psychologist to verify the presence or absence of psychotic symptoms. The interview schedule used in this study was the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime version (K-SADS) (Kaufman, et al., 1997) and the Structured Interview for Prodromal Syndromes (SIPS) (McGlashan, Miller, Woods, Hoffman, & Davidson, 2001). Both the child and one of his/her parents were interviewed.

There were a total of 37 children whose parents gave consent and participated in the clinical interview, which were classified post-interview to be 20 Con-C and 17
AR children. Not all of these subjects interviewed completed the imaging section of the study due to reasons such as: lack of consensual group classification, personal choice not to be scanned, did not complete the entire scan, and contra-indications to scanning. The AR participants were matched with healthy control children for gender, age, education, Wide Range of Achievement Test version four (WRAT-4) (Wilkinson & Robertson, 2006), which is a test of general scholastic ability, and family history of psychosis. The sample of children reported in the ABD analysis included two groups including 14 Con-C participants and 11 AR participants (Table 3.2).

3.3.2 fMRI task

All participants completed a motor GO/NOGO task to assess response inhibition during functional imaging. The task was programmed and displayed using E–prime 1.1 (Psychology Software Tools). There were two versions of the GO/NOGO task (see Chapter Two, Figure 2.1). For the ChSz study participants were presented with a serial stream of stimuli consisting of alternating X and Y letters every 1500ms which were presented in white against a black background. Each letter was presented for 1000ms and followed by a blank, black screen for a 500ms fixed inter-stimulus interval. Each block lasted 6 minutes and 17 seconds.

The ABD study participants were presented with the same stimuli as above but for 1000ms. Each letter was presented for 600ms and followed by a blank, black screen for a 400ms fixed inter-stimulus interval. Each block lasted 4 minutes and 42 seconds.

Participants from both studies were given the same instructions, which was to press a button for each stimulus while it was still present on the screen. NOGO stimuli, in which the target stimuli did not alternate (e.g., the fifth letter in the following sequence: X, Y, X, Y, Y, X...) required inhibition of the button response. For both
studies there were 450 GO and 50 randomly distributed NOGO stimuli presented over two runs.

Prior to scanning all participants signed study specific consent forms and were given a practice session of the task prior to scanning. All participants were then administered the Edinburgh Handedness Inventory.

### 3.3.3 Imaging acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a 30.5 cm internal diameter three-axis local gradient coil with insertable radiofrequency coils with transmit–receive capabilities. Participants viewed stimuli back-projected onto a 640 x 480 LCD screen at the head of the scanner bed viewed through a mirror mounted on the head coil in the participant’s line of sight. 180 high-resolution T1-weighted anatomic MPRAGE axial images (TE=3.8ms, TR=8.4ms, FOV 230 mm, thickness 0.9 mm, voxel size 0.9 mm x 0.9 mm x 0.9 mm, flip angle $\alpha=8^\circ$) were acquired for both studies.

Functional data from both studies were collected using a T2* weighted echo planar imaging (EPI) sequence that acquired 32 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE=35ms, TR=2000ms, FOV 224 mm, 64 mm x 64 mm matrix size). At the beginning of the functional scan four discarded acquisition scans (8s) were taken to allow for the stabilisation of T1 effects on the BOLD signal. Diffusion-weighted images were obtained using spin EPI (TE=52ms, TR=12343ms, FOV 224 mm, 150 mm matrix size) with 15 isotropically distributed orientations for the diffusion-sensitizing gradients at a $b$ value of 800 s/mm$^2$ and one $b=0$ image. Head movement was minimised with the use of foam pads adjusted at either side of the participants head.
3.3.4 fMRI analysis

Functional data was analyzed using the AFNI software package (Cox, 1996). The ChSz and ABD studies used the same processing pipeline. Following image reconstruction, the two 3-D time series (runs one and two) were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). The skull of the brains was removed using edge detection techniques. A deconvolution analysis calculated the hemodynamic response functions for successful inhibitions (STOPS) which were then modelled using a gamma-variate function (Murphy & Garavan, 2005). The modelling, using nonlinear regression, identified the best fitting gamma-variate function for each voxel. The area under the curve of this hemodynamic model was calculated for each voxel and expressed as a percentage of the area under the baseline (representing tonic task-related processes). These activation maps were resampled at 1 mm x 1 mm x 1 mm resolution, then standardised into Talairach space and (Talairach & Tournoux, 1988) and spatially blurred with a 4.2 mm full width half-maximum (FWHM) isotropic Gaussian kernel.

For the ChSz study separate Sz and Con-A activation maps for STOPS were determined with one-sample \( t \)-tests against the null hypothesis of even-related activity. Significant voxels were required to pass a voxelwise statistical threshold \([CON: t(14)=3.33, p<.005; Sz: t(14)=3.58, p<.005]\) and were required to be part of a larger 270\( \mu \)l cluster of contiguous significant voxels. For the ABD study separate AR and Con-C activation maps for STOPS were determined with one-sample \( t \)-tests against the null hypothesis of zero activation change. Significant voxels passed a voxelwise statistical threshold \([CON: t(13)=3.37, p<.005; AR: t(10)=3.58, p<.005]\) and were required to be part of a larger 273 \( \mu \)l cluster of contiguous significant voxels. The
cluster size thresholds were determined by means of a Monte Carlo simulation and resulted in 5% probability of a cluster surviving due to chance. To compare activations between the Sz and Con-A in the ChSz study and AR and Con-C groups in the ABD study, the thresholded activation maps were combined across groups for each study. The combined maps included the significant voxels of either group. This process was performed for the STOPS trials. The mean activation for clusters of activation in the combined maps were calculated for each participant and these functionally-defined, region-of-interest means were used for between-group independent \( t \)-tests. Detailed results from the ABD study can be found in Chapter Two and in Jacobson et al. (2010).

Exploratory analyses were performed to investigate the regions that differed between the Sz and AR groups (termed the PSY group) and the Con-A and Con-C groups (termed the CON group) during STOPS. A conjunction analysis (Nichols, Brett, Andersson, Wager, & Poline, 2005) was conducted which carried out separate \( t \)-tests directly contrasting ChSZ participants (Sz vs. Con-A) and ABD participants (AR vs. Con-C). These data were subjected to voxelwise thresholding at \( p<.005 \) and the cluster size criterion, which was obtained from the AlphaSim program carried out in AFNI, at \( p<.22 \) (which is the square root of \( p=.05 \)) yielded 214\( \mu l \) as the significant volume of cluster size. The thresholded data from the ChSz and ABD studies were then combined so that any voxels surviving would be significant. This analysis did not reveal any significant results. Furthermore, when the threshold was lowered to \( p<.1 \), there were still no clusters surviving thresholding.

A combined group analysis, which was subtly different from the conjunction analysis, was employed to reveal functional regions of interested that differed between groups. This analysis began with the data that resulted from the four separate
one-sample *t*-tests against a null hypothesis. The Sz and AR groups *t*-tests were each thresholded at *p*<.001 and then "ANDed" together such that a voxel only survived if significant in both groups. The same operation was carried out for the Con-A and Con-C groups to create the combined CON group map. Each of these combined maps, with a voxelwise significance of *p*<.001, were further thresholded and yielded a cluster size criterion at *p*<.224 (the square root of *p*=.05) of 133 µl of contiguous significant voxels. To compare activations between the CON and PSY datasets the thresholded maps were created using the same ROIstats program which was described for the ChSz study in this section. Results presented are from a post-hoc 2x2 ANOVA carried out in SPSS v.11.

### 3.3.5 VBM analysis

A VBM analysis is a whole-brain structural MRI analysis technique that is used for the characterization of regional cerebral volumes and the quantification of tissue concentration differences in anatomical MR images (Ashburner & Friston, 2000). Optimised VBM (Good, et al., 2001) was performed using FSL 4.0 VBM software (S. Smith, et al., 2004). First the participant’s structural T1 images were brain extracted. Then segmentation was performed in original space using FAST. The resultant GM partial volume images were then aligned to the MNI152 standard space using the affine registration tool FLIRT. The resulting images were then averaged to create a study-specific template to which the native GM images were nonlinearly re-registered. The registered partial volume images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 4.2 mm FWHM.
Voxelwise differences in GM between Con-A and Sz groups were tested with the non-parametric Mann-Whitney test. Significant voxels passed a voxelwise statistical threshold \([t(31)=2.81, p<.005]\) and were required to be part of a larger 376\(\mu\)l cluster of significant voxels. Voxelwise differences in GM between Con-C and AR groups were also tested with the non-parametric Mann-Whitney test. Significant voxels passed a voxelwise statistical threshold \([t(23)=2.81, p<.005]\) and were required to be part of a larger 320\(\mu\)l cluster of significant voxels.

A conjunction analysis was performed to explore the regions that showed a volumetric difference between the PSY group and the CON group. To compare volumetric differences between the CON and PSY datasets the output of the Mann-Whitney test for the ABD and ChSz studies were first thresholded a voxelwise statistical threshold of \(p<.07\) (the square root of \(p<.005\)) and were required to be part of a larger 320\(\mu\)l cluster of significant voxels. The ChSz and ABD studies thresholded maps were then ANDed together to create a map containing all of the regions differing in both the ABD and ChSz studies.

### 3.3.6 DTI analysis

DTI assesses WM connectivity in the brain by measuring the diffusion of water inside nerve fibre bundles. Fractional anisotropy (FA), which quantifies the degree of non-random diffusion, provides a proxy for WM integrity. Statistical analysis of the FA data was carried out using TBSS (Smith, et al., 2006).

Pre-processing involved the reconstruction of the diffusion tensors to create an FA map, which was generated using DTIFit within the FMRIB Diffusion Toolbox. The data were normalised to the FMRIB58_FA map, a standardised template of 58 high-resolution FA images averaged from healthy participants. The tract-based analysis approach creates a skeleton of tracts that participants have in common. For
each subject, a search is carried out along an invariant tract determined by the template and the voxel with the highest FA value is selected by comparing the FA value of the two nearest neighbours on each side in the direction perpendicular to the tract. No spatial blurring was required.

Voxelwise differences in FA between Con-A and Sz groups were tested with the non-parametric Mann-Whitney test. Significant voxels passed a voxelwise statistical threshold \( t(33)=3.29, p<.001 \) and were required to be part of a larger three \( \mu l \) cluster of significant voxels. (Note: \( p<.005 \) was also tested but produced 36 small separate clusters, so a more conservative threshold was used.) Voxelwise differences in FA between Con-C and AR groups were also tested with the non-parametric Mann-Whitney test. Significant voxels passed a voxelwise statistical threshold \( t(23)=2.81, p<.005 \) and were required to be part of a larger ten \( \mu l \) cluster of significant voxels (Note: Monte Carlo simulations revealed that four \( \mu l \) was sufficient to avoid false positives at \( p<.05 \), corrected, but this criterion produced an excessively high number of group differences due, presumably, to the absence of spatial blurring of the DTI data.)

A conjunction analysis was performed to explore the regions that showed an FA difference between the PSY group and the CON group. To compare FA differences between the CON and PSY datasets the output of the Mann-Whitney test for the ABD and ChSz studies were first thresholded a voxelwise statistical threshold of \( p<.07 \) (the square root of \( p<.005 \)) and were required to be part of a larger six \( \mu l \) cluster of significant voxels. The ChSz and ABD studies thresholded maps were then ANDed together to create a map containing all of the regions activated in both the ABD and ChSz studies.
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3.4 Results

3.4.1 Demographics

ChSz study

The sample consisted of 33 adults: 16 healthy adult controls with no history of a psychotic illness, Con-A, and 17 participants diagnosed with schizophrenia or schizoaffective disorder. Participants ranged in age from 25-61. All participants were Caucasians and native Irish citizens. Table 3.1 contains a summary of the demographic characteristics of the sample. No significant differences were found for age at date of scanning, gender, or education.

ABD study

The sample consisted of 25 children: 14 children with no history of psychotic symptoms, Con-C, and 11 children who had experienced a “definite” psychotic symptom, AR. Participants ranged in age from 11-13 years of age. All participants were Caucasian, native Irish citizens, and were attending their fifth or sixth year in mainstream primary schools in Dublin, Ireland. Table 3.2 contains a summary of the demographic characteristics of the sample. No significant group differences were found for age (in weeks) at date of scanning, gender, or family history of psychosis (Con-C: 14%, AR: 27%; $\chi^2=1.46$, $p=.48$). There was a six-month difference between the groups for years of education which reached statistical significance; however, education was included as a covariate in the subsequent analyses.

3.4.2 fMRI task performance

ChSz study

Table 3.3 reports the behavioural performance of the Con-A and Sz groups. The patients exhibited poorer inhibitory performance during the GO/NOGO task.
## Table 3.1

ChSz study demographics and clinical characteristics of the Sz patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sz (n=17)</th>
<th>Con-A (n=16)</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ± SD (in years)</td>
<td>42.8±11.3</td>
<td>44.4±10.7</td>
<td>$t(31)=.44$ $p=.67$</td>
</tr>
<tr>
<td>Age range (in years)</td>
<td>26-61</td>
<td>25-58</td>
<td></td>
</tr>
<tr>
<td>Gender (% male) (n=13)</td>
<td>76%</td>
<td>75% (n=12)</td>
<td>$\chi^2=.01$ $p=.92$</td>
</tr>
<tr>
<td>Education (in years)</td>
<td>12.4±2.3</td>
<td>13.6±2.1</td>
<td>$\chi^2=11.3$ $p=.23$</td>
</tr>
<tr>
<td>Education range (in years)</td>
<td>8-16</td>
<td>11-18</td>
<td></td>
</tr>
</tbody>
</table>

**Clinical data**

- Age of onset: 22.5±4.6
- Age of onset range (in years): 14-33
- Illness duration: 20.3±11.4
- Illness duration range (in years): 6-43
- PANSS positive: 24.8±14.9
- PANSS positive range: 9-55
- PANSS negative: 19.2±5.1
- PANSS negative range: 11-28

**Diagnostic information**

- Schizophrenia-Paranoid (n): 13
- Schizoaffective (n): 4

**Anti-psychotic medication**

- Typical\(^a\): 4
- Atypical\(^b\): 12
- Both: 1

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\(^a\)Depixol (n=1), Fluphenazine (n=1), Largactil (n=1), Zuclopenthixol (n=2), \(^b\)Chlorpromazine (n=1), Risperidol (n=5), Olanzapine (n=7), Clozapine (n=3), Amisulpride (n=2).

Note: Some Sz patients were medicated with more than one atypical anti-psychotic medication (n=5).
relative to the controls, but this difference was not significant. There was, however, a significant difference in reaction time (RT) between groups for GO trials, which was subsequently entered as a covariate in the subsequent fMRI analysis.

Table 3.3
ChSz study behavioural performance during the GO/NOGO task.

<table>
<thead>
<tr>
<th>Group</th>
<th>Accuracy</th>
<th>Statistic</th>
<th>RT</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con-A (n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>43±7</td>
<td>t(28)=1.78</td>
<td>451±48</td>
<td>t(28)=3.23</td>
</tr>
<tr>
<td>Sz (n=15)</td>
<td></td>
<td>p=.09</td>
<td></td>
<td>p=.003</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>38±10</td>
<td></td>
<td>527±78</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4 reports the behavioural performance of the Con-C and AR groups. The at-risk children exhibited poorer inhibitory performance during the GO/NOGO task relative to the controls, which was similar in the divergence found between the Sz and Con groups, but this difference was not significant. RT performance was comparable in both Con-C and AR groups.
### Table 3.4

<table>
<thead>
<tr>
<th>Group</th>
<th>Accuracy</th>
<th>Statistic</th>
<th>RT</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con-C (n= 14)</td>
<td>21±8</td>
<td>t(23)=1.83</td>
<td>684±47</td>
<td>t(23)=.53</td>
</tr>
<tr>
<td>AR (n= 11)</td>
<td>p=.08</td>
<td></td>
<td></td>
<td>p=.6</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>15±8</td>
<td></td>
<td>670±90</td>
<td></td>
</tr>
</tbody>
</table>

#### 3.4.3 fMRI activation

**ChSz study**

The activated regions during STOPS were similar to those identified in other imaging studies of GO/NOGO tasks (Garavan, et al., 1999). For STOPS, the OR maps revealed activations in the DLPFC, VLPFC, cingulate gyrus, basal ganglia, and parietal lobe. The Sz group showed marked reductions in activation during inhibition trials in five regions when compared to the Con-A group. The results of this ANOVA are presented in Table 3.5 and Figure 3.1. These regions included the right IFG \(F(3,28)=5.29, p<.01\), right caudate \(F(3,28)=3.09, p=.04\), left caudate \(F(3,28)=3.99, p=.02\), right posterior cingulate \(F(3,28)=4.42, p<.01\), left hypothalamus \(F(3,28)=5.08, p<.01\). The Sz patients showed one region of hyperactivation that fell within the left superior frontal gyrus \(F(3,28)=5.94, p<.005\).

**ABD study**

Table 3.6 presents the regions of activity found in the Con-C and AR children from the ABD study. The activation patterns of the groups differed with greater right-sided activation for STOPS in the Con-C group and more left-sided activation in the AR group (see Chapter Two, Figure 2.3). The Con-C group had significantly greater activation in six regions compared to the AR group (see Chapter Two, Figure 2.4). These areas were predominantly right lateralised and included the right IFG, right side, and other relevant regions.
### Table 3.5
ChSz study regions of activation during STOPS.
These clusters were derived from whole-brain, voxelwise analysis ($p<.05$, corrected). Results from the post-hoc ANOVA ($p<.05$) of brain regions showing between group differences are listed in the far right column.

<table>
<thead>
<tr>
<th>Region</th>
<th>Side</th>
<th>BA</th>
<th>Vol. ($\mu l$)</th>
<th>$x$</th>
<th>$y$</th>
<th>$z$</th>
<th>L</th>
<th>P</th>
<th>I</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate gyrus</td>
<td>R</td>
<td>32</td>
<td>5683</td>
<td>5</td>
<td>46</td>
<td>9</td>
<td>2</td>
<td>37</td>
<td>34</td>
<td>Con-A&gt;Sz</td>
</tr>
<tr>
<td>Posterior cingulate gyrus**</td>
<td>R</td>
<td>31</td>
<td>711</td>
<td>2</td>
<td>-37</td>
<td>34</td>
<td>4</td>
<td>14</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Cingulate gyrus</td>
<td>R</td>
<td>32</td>
<td>771</td>
<td>4</td>
<td>14</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>R</td>
<td>47</td>
<td>12801</td>
<td>41</td>
<td>14</td>
<td>-11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior frontal gyrus**</td>
<td>R</td>
<td>46</td>
<td>671</td>
<td>54</td>
<td>31</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>Con-A&gt;Sz</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>L</td>
<td>47</td>
<td>5178</td>
<td>-31</td>
<td>16</td>
<td>-29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>R</td>
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*p<.05; **p<.01; ***p<.005
precuneus, right lentiform nucleus, right middle temporal gyrus, left middle temporal gyrus and left claustrum. There were four brain regions with greater activity in the AR group. These were left lateralised and included the left medial frontal gyrus/ACC (BA32), left ACC (BA24), left superior frontal gyrus, and left precentral gyrus (see Chapter Two, Figure 2.5).

**Combined group**

The STOPS maps revealed a total of activations within 20 regions for both the PSY and CON groups, within expected regions, mainly within the right hemisphere in the DLPFC, temporal and parietal lobes (see Table 3.7 and Figure 3.2). In line with the literature on response inhibition and deficits in schizophrenia, significant group differences were observed in largely right frontal regions. The CON group showed greater activity within the IFG [t(53)=2.09, \( p=.04 \)], right superior frontal gyrus [t(53)=2.68, \( p=.01 \)], left middle temporal gyrus [t(53)=2.01, \( p<.05 \)], and left claustrum
Chapter Three

Cross-sectional study of the risk for psychosis

\[ t(53)=3.08, p=.003 \]. The PSY group showed an increase in activity with one region, left precuneus \[ t(53)=-3.07, p=.003 \].

Table 3.6

**ABD study regions of activation during STOPS.**

These clusters were derived from whole-brain, voxelwise analysis \( p<.05 \), corrected). Results from the post-hoc ANOVA \( p<.05 \) of brain regions showing between group differences are listed in the far right column.

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<th>( y )</th>
<th>( z )</th>
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*p<.05; **p<.01; ***p<.005
Figure 3.2. GO/NOGO combined group analysis results during successful inhibitions (STOPS). Axial images display the 20 clusters of activation, represented in the various coloured clusters, co-occurring in the adult and children controls (CON) group and in the at-risk and schizophrenia patients (PSY) group ($p<0.05$, corrected). Activations occurred mainly in the right frontal hemisphere and were more active in the CON group.
Table 3.7
GO/NOGO task combined group analysis results.
These clusters were derived from whole-brain, voxelwise analysis ($p<.05$, corrected). Results from the post-hoc ANOVA ($p<.05$) of brain regions showing between group differences are listed in the far right column.

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<th>y</th>
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*p<.05; **p<.01; ***p<.005
Chapter Three

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3.4.4 VBM

ChSz study

The Sz patients revealed significant between-group differences in four regions of the brain. The Sz group showed reductions in GM volumes within two regions: right posterior cingulate at Talaraich coordinates (872μl; LPI: 14, -61, 16) and left middle occipital gyrus (424μl; -30, -92, 12). The Sz patients revealed an increase in GM within two regions: left angular gyrus (448μl; -42, -72, 38) (Figure 3.3) and left STG (408μl; -64, -18, -8) (Figure 3.4).

ABD study

The AR group had significantly larger GM volumes in four regions: left middle temporal gyrus (BA21) at Talaraich coordinates (117μl; LPI: -60, -48, 0), left angular gyrus (BA39) (344μl; -50, -74, 36) (Figure 3.3), left STG (BA38) (376μl; -54, 16, -18) (Figure 3.4), and right orbitofrontal gyrus (BA 11) (584μl; 18, 36, -26) when compared to the CON Group (see Chapter Two, Figures 2.6A-D). The CON group had significantly larger GM volume in the left inferior temporal gyrus (BA 20) (376μl; -68,-28, -14) (see Chapter Two, Figure 2.6E).

Combined group

There were two regions that did not appear in the combined group analysis, due to the lack of direct overlap in GM; however, these regions fell in close proximity to one another and may be of interest. Figure 3.3 presents the left angular gyrus and Figure 3.4 presents the left STG which both revealed an increase in GM for Sz>Con-A and AR>Con-C groups for the corresponding ChSz and ABD studies.

The group conjunction analysis showed reduced GM volume in the PSY group within only one region, in the right insular cortex (684 μl; 44, 4, -4) (Figure 3.5).
Figure 3.3. ChSz and ABD studies VBM results. In the ChSz study schizophrenia patients showed an increase in grey matter within the left angular gyrus (red) (LPI: -42, -72, 38). In the ABD study the at-risk children also revealed an increase in grey matter within the angular gyrus (yellow) (-50, -74, 36).

Figure 3.4. ChSz and ABD studies VBM results. In the ChSz study schizophrenia patients showed an increase in grey matter within the left superior temporal gyrus (red) (LPI: -64, -18, -8). In the ABD study the at-risk children also revealed an increase in grey matter within the superior temporal gyrus (yellow) (-54, -16, -18).
Figure 3.5. Conjunction analysis VBM results. There was one region, which fell in the right insula (blue), which showed a volumetric reduction in the psychosis group (PSY) relative to healthy controls (CON) (LPI: 44, 4, -4).

3.4.5 DTI

ChSz study

Table 3.8 reports the ChSz study DTI analysis revealed FA differences that were reduced in the Sz group in nine regions and increased in only one region.

ABD study

Two participants were not included in the DTI analysis (1 CON subject due to excessive head movement and one AR subject who did not complete the entire MRI session due to fatigue).

Table 3.9 reports the three regions that revealed between-group differences. Reduced FA values in the AR group relative to the CON group fell along the inferior fronto-occipital fasciculus, within the lingual gyrus; the cingulum bundle, within the left parahippocampal gyrus; and the inferior longitudinal fasciculus, within the left STG (see Chapter Two, Figure 2.7).
Table 3.8
ChSz study DTI FA analysis results.
These clusters were derived from whole-brain, voxelwise analysis ($p<.05$, corrected). These results are from the post-hoc ANOVA ($p<.05$) of the brain regions revealing between group differences.

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</tr>
<tr>
<td>Putamen</td>
<td>R</td>
<td>8</td>
<td>20</td>
<td>-15</td>
<td>-1</td>
<td></td>
<td>Sz&gt;Con-A</td>
</tr>
</tbody>
</table>

Table 3.9
ABD study DTI FA analysis results.
These clusters were derived from whole-brain, voxelwise analysis ($p<.05$, corrected). These results are from the post-hoc ANOVA ($p<.05$) of the brain regions revealing between group differences.

<table>
<thead>
<tr>
<th>Fibre tract and region</th>
<th>Side</th>
<th>BA</th>
<th>Vol. (µl)</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Direction</th>
</tr>
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<tr>
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<td>91</td>
<td>0</td>
<td>Con-C&gt;AR</td>
</tr>
<tr>
<td>Cingulum bundle</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Con-C&gt;AR</td>
</tr>
<tr>
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<td>-27</td>
<td>-20</td>
<td>-30</td>
<td></td>
<td>Con-C&gt;AR</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus</td>
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<td></td>
<td></td>
<td></td>
<td>Con-C&gt;AR</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
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<td>-37</td>
<td>-34</td>
<td>-6</td>
<td></td>
<td>Con-C&gt;AR</td>
</tr>
</tbody>
</table>
Conjunction

This analysis did not reveal any regional FA reductions within the frontal or temporal lobes. Table 3.10 describes the 13 regions that showed significant group differences in FA, nine of which showed FA reductions in the PSY group and four regions of FA increase in the PSY group.

Table 3.10
DTI FA conjunction analysis results.
These clusters were derived from whole-brain, voxelwise analysis \( (p<.05, \text{corrected}) \). These results are from the post-hoc ANOVA \( (p<.05) \) of the brain regions revealing between group differences.

<table>
<thead>
<tr>
<th>Fibre tract and region</th>
<th>Side</th>
<th>BA</th>
<th>Vol. (μl)</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Direction</th>
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<tr>
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<td>36</td>
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<td>32</td>
<td>CON&gt;PSY</td>
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<tr>
<td>Precentral gyrus</td>
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<td>-38</td>
<td>-7</td>
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<td>15</td>
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<td>20</td>
<td>-10</td>
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</tr>
<tr>
<td>Middle cerebellar peduncle</td>
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<td>R</td>
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<td>-26</td>
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</tr>
<tr>
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<tr>
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</table>
3.5 Discussion

3.5.1 fMRI

Separate studies

The GO/NOGO task that set out to elicit regions involved in the inhibition of a pre-potent response revealed similar patterns of activation across the two studies. Regions activated included frontal, temporal, and parietal cortex as well as the cingulate, cerebellum, claustrum, and precuneus. However, the Sz patients revealed a unique pattern of activation which was different from their controls and also the children in the ABD study. Individuals with the disease revealed hypoactivation within the bilateral caudate, left hypothalamus, and right posterior cingulate whereas the AR children revealed significant hypoactivations not observed in the Sz patients within bilateral middle temporal gyri, right lentiform nucleus, right precuneus, and left claustrum. Additionally, the AR children produced hyperactivity within the left ACC, left medial frontal gyrus, and left precentral gyrus. Despite these different patterns of group difference, a common set of differences between the PSY and CON groups were found in the combined group analysis. These regions included the right IFG, left superior frontal gyrus, middle temporal gyrus and claustrum, which will be discussed in-depth in a following section.

While there were regions that differed in activity between the psychosis groups, two of these regions serve similar functional operations in the brain. The hypoactivity observed in the right caudate in the Sz patients and that seen in the right lentiform nucleus in the AR children both fall within the dorsal striatum. These two regions are functionally similar in that they are both involved in voluntary movements (Snell, 2006). Activity in the dorsal striatum is initiated by information received from the premotor and supplementary motor areas, the motor cortex, and the primary
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sensory cortex (Snell, 2006). In regards to communication pathways, a simplified model states that the striatum receives dopaminergic input from the substantia nigra, than the inhibitory neurotransmitter GABA is produced by the dorsal striatum, which projects to the global pallidus and back to motor areas (Snell, 2006). An increase in global pallidus activity is believed to play an important role in the preparatory behaviour that enables the limbs of the body to be placed in the appropriate positions, before the primary cortex can allow activation of movements of the hands and feet (Snell, 2006). Therefore, the hypoactivity we found in the psychosis groups in the dorsal striatum during this motor task of response inhibition may indicate disturbances in DA production from the substantia nigra resulting in the slowed neuromotor movements, which have been shown in previous studies of individuals at-risk who experience psychotic-like symptoms (Cannon, et al., 2002b). I also found slower reactions times within my risk cohort during the neuropsychological testing session for the Purdue Pegboard Task (see Appendix Five, Table 1). The Sz group also exhibited significantly slower RTs and the AR group had a trend for slower RTs than controls during the GO trials of the GO/NOGO task.

Reduced activation in the left hypothalamus was observed in the Sz patients, but not in the AR children. This region, which lies at the centre of the limbic system, integrates information received by the nervous system to bring about the physical expression of emotion (i.e. rise in blood pressure and heart rate) (Snell, 2006). The hypothalamus connects directly to the pituitary gland to regulate hormone production in the endocrine glands. Suggested explanations of the emotional and behavioural abnormalities observed in the risk for schizophrenia are drawn from the diathesis-stress model of schizophrenia (Hans & Marcus, 1987). Walker and Diforio (1997) took the model a step further to develop the neural diathesis-stress model, which
postulated that a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which regulates physiological stress in mammals, could trigger the expression of dysfunctional neural circuits that subserve psychotic symptoms. This theory does not assume that frequency of stressful life events contribute to the development of psychosis, but rather links stress with the internal perception of the event. In other words, it does not seem to be the amount of psychosocial stressors that plays a role in schizophrenia per se, but rather the salience that is given to the event that is perceived as stressful to the individual. When a biological response occurs in the presence of the stressor, the HPA axis is activated and triggers the neural substrates for psychosis (Walker, Mittal, & Tessner, 2008). Increased levels of the stress hormone cortisol have been found within adults who have schizophrenia (Altamura, Guercetti, & Percudani, 1989; Corcoran, et al., 2003). Romeo and McEwen (2006) have stated that pubertal maturation has been shown to have a marked influence on HPA axis plasticity, specifically relating to neural activations elicited by acute and chronic stressors. Additionally, they found that regions still maturing during adolescence, including the PFC, hippocampus and amygdala, are the most sensitive to the effects of cortisol. Therefore, the difference in developmental stages between the children and adult psychosis groups may be the explanation behind the relative lack of task induced hypothalamic activity in children. Furthermore, deficits in the hypothalamus and subsequent increases of cortisol may be a product of the onset of the disease, and therefore, not observable before the transition to the illness. This idea is supported by a study of individuals at ultra-high risk (Mean age: 18 years), who subsequently developed psychosis and who had lower levels of plasma cortisol levels upon admission to the study (two-year follow-up period) than those who did not develop
the illness (Thompson, et al., 2007). In this study higher levels of cortisol were also correlated with increased measures of depression and anxiety.

The final region that showed a reduction in activity for the Sz patients but not in those at-risk was in the right posterior cingulate. This finding is in line with Laurens and colleagues (2005) who found an increase in the posterior cingulate cortex during response inhibition in healthy controls. The posterior cingulate has been shown to possess functionality which appears to be involved in motivational biasing of external stimulus processing during goal-directed processing (Mesulam, Nobre, Kim, Parrish, & Gitelman, 2001). This is in contrast to the ACC, which is commonly activated in tasks involving response conflict, due to its role in conflict monitoring (van Veen, Cohen, Botvinick, Stenger, & Carter, 2001). Another possible explanation for the lack of posterior cingulate functioning is that the reduced activity in the Sz patients may reflect their overall blunted motivational state and indifference towards completing this task at hand. A structural explanation for the reduced posterior cingulate functioning may be the volumetric reduction in right posterior cingulate GM in the Sz patient group, which was not observed in the AR group.

**Combined group**

The combined group analysis set out to explore regions that showed group differences between the two psychosis groups (PSY), one with the illness and the other with increased vulnerability for developing schizophrenia, compared to their matched adult and children control groups (CON). The regions activated during STOPS included cingulate, frontal, temporal, parietal and subcortical regions. However, group differences revealed hypofrontality in the PSY group, specifically within the right IFG, superior frontal gyrus (SFG). Other reductions were found in the left middle temporal gyrus and left claustrum. Hyperactivity in the PSY group was
found only in the left precuneus. The regions in the IFG and middle temporal lobe showing hypoactivity in the PSY group are well-known to be involved in working memory in healthy adults (Haxby, Petit, Ungerleider, & Courtney, 2000; Jonides, et al., 1993). With regard to the presence of psychotic symptoms, abnormalities in both the right IFG (Sommer, et al., 2008) and the middle temporal lobe (Shergill, et al., 2004; Zhang, et al., 2008) have been implicated as possible underlying neurobiological mechanisms contributing to the presence of auditory verbal hallucinations.

PFC

During successful inhibitions both the right IFG and SFG showed activation in all groups; however, the PSY group reported less functional activation in both regions relative to the CON group. The PFC is regarded as a brain region of higher-order cognitive processing, in which representation and execution of actions takes place (Fuster, 2000). It is also the last cortical region to develop (Giedd, et al., 1999). The IFG (BA 47) is referred to as the ventral lateral PFC (VLPFC) and has been shown to be active in the right hemisphere during fMRI tasks of behavioural inhibition using GO/NOGO tasks (Garavan, et al., 1999; Konishi, Nakajima, Uchida, Sekihara, et al., 1998), reversal learning (Cools, Clark, Owen, & Robbins, 2002), and in response to negative feedback during the Wisconsin Card Sorting Task (Monchi, Petrides, Petre, Worsley, & Dagher, 2001). Previous reports of hypoactivity in the VLPFC in schizophrenia patients have been found during tasks of response inhibition (Kaladjian, et al., 2007) and working memory (Stevens, Goldman-Rakic, Gore, Fulbright, & Wexler, 1998). A single photon emission computed tomography (SPECT) study of patients with impulsivity disorders also revealed less brain perfusion in the right VLPFC in the patients with borderline or anti-social personality disorder (Goethals, et
Together these results indicate reductions in right VLPFC functioning may be related to an inability to recruit this region of the brain required for inhibition, which in turn, may manifest itself in the impulsive behaviours and actions commonly observed in schizophrenia patients.

The SFG (BA 9) is part of the DLPFC, which is the last region of the brain to complete GM maturation (Gogtay, et al., 2004) and myelination (Jernigan & Tallal, 1990). This region has been found to show reduced cerebral blood flow in unmedicated schizophrenia patients (Weinberger, et al., 1986). The VLPFC and DLPFC are two PFC regions which have been shown to be activated differentially depending on the operations involved in the cognitive task. Nee et al. (2007) examined imaging studies of various cognitive control tasks, including GO/NOGO, Flanker, Stroop, and stimulus-response compatibility tasks. This meta-analysis found that the left DLPFC was actively involved in response selection, during the Stroop task and right DLPFC during the Flanker task, whereas the right VLPFC was recruited during the response inhibition during the GO/NOGO task. A further functional differentiation has been found in schizophrenia patients who have shown deficits in the VLPFC during tasks of working memory maintenance, while the DLPFC shows deficits during tasks involving working memory manipulation (Minzenberg, Laird, Thelen, Carter, & Glahn, 2009). I observed functional reductions in the right hemisphere in both of these regions during the GO/NOGO task in the PSY group, revealing a deficit in the PSY group's ability to both withhold a response and carry out that goal-directed behaviour. A meta-analysis of 27 fMRI and PET studies, which all reported DLPFC activation during working memory tasks, found that when performance was controlled for schizophrenia patients showed a significant reduction in the right DLPFC (Van Snellenberg, Torres, & Thornton, 2006).
One of the most important findings of the present study is that the PSY group showed reduced neural activity in a number of brain regions despite similar performance levels on the GO/NOGO task, although a minimal performance difference was found in the Sz patients, but controlled for in post-hoc tests. This indicates that there is an underlying functional deficit in schizophrenia patients and that activation patterns are not secondary to behavioural abilities. One explanation for the right lateralised hypofrontality may be that the schizophrenia patients recruit a wider network of left hemisphere brain regions in response to their inability to sufficiently recruit right frontal regions. However, the PFC does not act in isolation and its functions are facilitated by connections to an extensive network of other cerebral structures. Patterns of functional activation in brain regions connected to the PFC may reveal difference in neural networks being used in the healthy controls versus those used in the psychosis group.

**Middle temporal gyrus**

We found a reduction in activity within the left middle temporal lobe in the PSY group. The left temporal lobe contains the primary auditory cortex and also Wernicke's area, which are regions involved with semantics, language, speech, and vision (Snell, 2006). Therefore, the temporal lobe can be conceptualised as a central hub where the convergence of semantic, verbal, visual, and auditory associations co-occur, rendering this region of the cortex as an area of certain relevance to the behavioural deficits and positive symptoms observed in schizophrenia. Structurally, the temporal lobe is one of the most commonly reported regions of volumetric reduction in schizophrenia patients (Shenton, Dickey, Frumin, & McCarley, 2001; Shenton, et al., 1992). Crow (1990) found that left lateralised temporal structural deficits play a role in abnormalities in speech and insight, incongruent emotional
expression, severe paranoia, and auditory and visual hallucinations in schizophrenia patients. Temporal lobe dysfunctions in schizophrenia patients have been found for the P300 event-related potential (ERP) during an auditory modulation task (Ford, Roth, Menon, & Pfefferbaum, 1999), which was correlated with increased symptom severity. The temporal lobe has been strongly related to the presence of the hallmark positive symptoms associated with schizophrenia, such as auditory and visual hallucinations (Pearlson, 1997). Shergill and colleagues (2004) conducted an fMRI study with schizophrenia patients and found increased activity in bilateral temporal gyri and left insula during auditory verbal hallucinations. Zhang and colleagues (2008) used a similar paradigm and found hyperactivity within Wernicke's area for the schizophrenia patients, but not in controls. Additionally, a meta-analysis of the executive Stroop task revealed controls recruit the left middle temporal gyrus during successful trials of this response conflict task, but the region remains hypoactive in the schizophrenia patients (Minzenberg, Laird, Thelen, Carter, & Glahn, 2009). We found that temporal lobe deficits in the children at-risk is in contrast to previous research which concluded that temporal deficits in schizophrenia do not emerge until later in the course of the illness (Salisbury, et al., 2002). This would fall in line with theories of normal brain development, which have found the temporal lobes do not fully mature until age 16 (Giedd, et al., 1999). The current finding of reduced temporal functioning in both psychosis groups suggests that this regional dysfunction may be related to the presence of positive psychotic-like symptoms in an individual, which is identifiable before the full neuronal maturation of this region has occurred.

Clastrum

The PSY group revealed reduced activity within the left clastrum. Neuroanatomically, the subcortical clastrum structure is hidden between the surface
of the neocortex, just below the insula and above the putamen and connected by WM tracts to the external capsule. Little is actually known about the function of the human claustrum. A study of the cat claustrum showed that this region is involved in a bi-directional loop which receives glutamatergic input from the primary visual cortex (V1) and auditory cortex, and is projected back to visual area V4, along the ventral stream pathway passing through the inferior temporal lobe (Olson & Graybiel, 1980). The claustrum also has widespread connections to nearly every region of the cortex, including the frontal lobe (PFC, motor cortices, and cingulate), medial temporal lobe (hippocampus), occipital lobe, and posterior parietal cortex (Tanne-Gariépy, Boussaoud, & Rouiller, 2002).

With regards to its function, a PET study revealed activation in the claustrum and insular regions during a task of cross-model sensory matching (Hadjikhani & Roland, 1998). This study supports the hypothesis of the claustrum’s role in cross-modal processing (Calvert, 2001; Ettlinger & Wilson, 1990). Crick and Koch (2005, p.1276) have analogised the claustrum as “a conductor coordinating a group of players in the orchestra, the various cortical regions. Without the conductor, the players can still play but they fall increasingly out of synchrony with each other. The result is a cacophony of sounds.” This leads to the belief that a deficit in claustrum functioning would fall in line with theories of schizophrenia patients having an impaired ability to simultaneously integrate objects and events that co-occur in the real world to allow the subject to consciously perceive the entirety of an event or object and respond appropriately. In laboratory settings this ability can be tested using context processing tasks, which have been shown to be deficient in schizophrenia patients and unaffected relatives (Carter, MacDonald, Ross, & Stenger, 2001; Macdonald, Thermenos, Barch, & Seidman, 2008). Additionally, due to the highly
interconnected nature of the claustrum, the disconnectivity theory of schizophrenia would suggest that a deficit in this region, which has widespread and reciprocal connectivity with almost the entire cerebrum, would suggest compromised optimal intercortical communication, thus accounting for the psychotic and cognitive manifestations (Weinberger, Aloia, Goldberg, & Berman, 1994).

Another unique aspect of the claustrum is the presence of spiny interneuron gap junctions. These gap junctions provide a low-resistance, bi-directional, electrical pathway between neurons (Crick & Koch, 2005) that connects specifically to inhibitory neurons (Gibson, Beierlein, & Connors, 1999). These gap junctions cause target membranes to depolarise in one interneuron and spread to others, creating a cascade of synchronous neural firing of interneurons. This synchronous firing may be the underlying neurobiological mechanism subserving cross-modal integration in the claustrum. A meta-analysis of executive functioning in schizophrenia patients found reduced activity within the left claustrum compared to healthy controls (Minzenberg, Laird, Thelen, Carter, & Glahn, 2009). Therefore, since the claustrum has an integral role in inter-cortical connectivity a dysfunction in this region may also give rise to the presence of positive symptoms which arise from a misattribution of internal and external events.

**Precuneus**

Anatomically, the precuneus is located in the posterior region of the brain, just dorsal to the parieto-occipital sulcus in the parietal lobe. The precuneus has neuronal connections with premotor regions and is an integral component of the sensorimotor network in the brain (Luppino, Murata, Govoni, & Matelli, 1999; Petrides & Pandya, 1984), which subserves spatially guided behaviours and visuo-spatial processing (Selemon & Goldman-Rakic, 1988). Resting-state functional connectivity analyses
have revealed correlations in activation patterns between the precuneus/posterior
cingulate cortex and medial prefrontal cortex, which comprise the default-mode
network (van den Heuvel, Mandl, Luigjes, & Hulshoff Pol, 2008). These regions have
been collectively comprised as the "task-negative" network of the brain, meaning they
are more active during the resting-state (i.e. in the absence of performing a task) than
during cognitive tasks (Fox, et al., 2005). The increased activity during rest in the
precuneus may be due to the fact that the precuneus has the highest resting metabolic
rates in the brain, consuming over 35% more glucose than any other region of the
cortex (Gusnard & Raichle, 2001). Since glucose metabolism is often accompanied by
increased cerebral blood flow (Fox, Mintun, Reiman, & Raichle, 1988) this may be
why activity in this region appears during fMRI scans. Further research during in the
resting-state and during goal-directed processing tasks has revealed that during rest
the precuneus produced remarkably high metabolic rates, but revealed deactivations
during the cognitive task (Cavanna & Trimble, 2006). In regards to mechanisms
underlying the neural inefficiency hypothesis, the ability to deactivate or suppress this
region and in turn produce task-relevant neural activity in other regions of the brain,
such as the DLPFC, is crucial to efficiently carrying out cognitive processing. The
present results revealed that the left precuneus showed a greater level of deactivation
in the CON group compared to the PSY group. However, in actuality it was the AR
group that was driving this effect. All of the Sz patients (n= 15) successfully
deactivated this region to complete the task, whereas only one AR subject was able to
deactivate this hypothesised task-negative region (Figure 3.6).
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Figure 3.6. Results from the combined group analysis \((p<.05,\text{ corrected})\) of mean activation in the precuneus during STOPS. The at-risk (AR) group revealed the greatest amount of positive activity in the precuneus relative to baseline, whereas the schizophrenia (Sz) group had the highest amount of deactivation in this region and the control groups did not engage this region during the task.

The observed hyperactivity in the normally task-negative region of the precuneus within the AR group could be explained by the elevated activity typical of the resting-state network which is enhanced to gather available interconnected cortical resources to complete the task at hand. In controls subjects on the other hand, they do not seem to engage this region during the task suggesting that this passive network in healthy controls is suppressed during the complex goal-directed behaviour to allow higher order cognitive processes to take over in more efficient cortical regions.

Connectivity pathways in the brain provide the basis of the neural network forming the subcortical and posterior sensorimotor system, which has been shown to
be recruited in early life cognitive functioning (Greenough, 1975). The hyperactivity found in the precuneus in the AR group could be interpreted as this group relying on less developmentally mature regions, as opposed to the frontal cortex, to complete the task. Rubia and colleagues (2000) have suggested that the mature task related network of the brains, which includes the frontal lobes, is recruited when task-related demands or difficulty surpass the abilities of the more immature brain networks, of the posterior regions. The increase in posterior region functioning in the AR group displays an over reliance on a region of the brain which is developmentally inferior to more frontal regions. Additionally, a recent neuroimaging study reported that during a response inhibition task children activated more diffuse and task-irrelevant brain areas than adults, which included the precuneus (Braet, et al., 2009). However, since we are observing an increase in posterior functioning in both the child and adult psychosis groups, this may reveal that this brain region has reached its highest level of attainable maturation and the PSY group therefore recruits this region to compensate for less developed frontal regions. Other neuroimaging studies have reported an increase in parietal lobe functioning in schizophrenia patients compared to healthy controls (Curtis, et al., 1998; Paulus, et al., 2002; Spence, et al., 1997). A compensatory strategy of recruiting the parietal brain regions instead of the ACC, was also found in the Edinburgh High Risk Study and thought to be indicative of a trait-related risk for psychosis (Whalley, et al., 2004). They found increased activity during a verbal fluency task in the parietal lobe and decreased prefrontal activity in individuals at a high genetic risk who also displayed isolated psychotic symptoms. Individuals who were at genetic risk but not experiencing psychotic symptoms did not show increased activation within the parietal lobe. Additionally, research using single photon emission computed tomography (SPECT) to assess cerebral blood flow in individuals
with a mild cognitive impairment found hyperprofusion in the precuneus in those who converted to Alzheimer’s disease, indicating a commonality in another patient’s group with severe cognitive impairments. Additionally structural reductions have been found in the precuneus in at-risk subjects who later transition to psychosis (Borgwardt, et al., 2007), whereas medicated, first-episode patients had a slightly greater volume of GM in the precuneus than the at-risk subjects.

The observation that the greatest deactivation in the precuneus was found in the schizophrenia patients at the time of scan were all on antipsychotic medication implies that activations in the precuneus may be mediated by pharmacologic intervention. The rationale behind this theory is as follows. Atypical antipsychotic drugs used in the treatment of schizophrenia are known to have opposing effects on DA transmission in the brain. Some examples are Risperidal and methylphenidate. In our scanning sample 80% (n= 12) of patients were medicated with atypical antipsychotics at the time of treatment, mainly Risperidal. These drugs are known to block DA reuptake, which subsequently increases the level of DA neurotransmitters in the synapse (Volkow, et al., 1998). However, blocking this reuptake stimulates the release of DA into the synapse causing the magnitude of DA release to increase therefore increasing the salience attributed to a given stimuli. The dopamine hypothesis put forth by Kapur et al. (2005), posit that aberrant DA transmission leads to salience misattribution of irrelevant events in schizophrenia patients. Therefore, the antipsychotics play an important role in modulating this effect. There is also evidence that DA reuptake blockers reduce the amount of glucose metabolism during cognitive tasks by up to 50%, allowing the brain to become more focused and efficient in its mental processing, and increasing activity in the PFC and hippocampus (Volkow, et al., 2008). Therefore, in the AR individuals they may not reveal the same patterns of
activity in the precuneus due to not having the stable pharmacological intervention of the Sz patients, which affects DA transmission or glucose metabolism.

In summary, the hyperactivity found in the precuneus in the AR subjects reveals a potential biomarker in the brain of the detection of psychosis which is notably found in the risk stage, but not in those with the disease who are medicated. Therefore, this region could be an important locus of therapeutic intervention during the risk stage, which seems to show improved functioning in this area with the treatment of antipsychotic medication.

Among all of the regions that revealed group differences during the GO/NOGO task the most robust region was the right VLPFC region. This brain region, which is crucial to inhibiting irrelevant information and focusing attention showed global reductions in activity in both the at-risk and patient groups. The inefficiency hypothesis would suggest that when the hypofrontality is observed in this region the less efficient but nonetheless compensatory left superior frontal region comes on-line to support successful completion of the task. From a neurodevelopmental perspective the fact the these patterns of frontal activity are observed regardless of age may suggest that these PFC regions never reach full maturity in order to perform at the most efficient levels in the brains of those who go on to develop schizophrenia.

### 3.5.2 VBM

**Separate studies**

Studies examining the neurodegeneration hypothesis of schizophrenia have revealed a pattern of cortical GM loss which moves from posterior to anterior brain regions (Gogtay, et al., 2004; Thompson, et al., 2001; Vidal, et al., 2006). In studies of first-episode and chronic schizophrenia patients prefrontal GM VBM reductions
have been found to be a central feature (Gur, et al., 1998; Shenton, Duckey, Frumin, & McCarley, 2001). However, I did not find GM reductions in the frontal lobe in the either psychosis group. However, AR subjects revealed a GM increase in the right orbitofrontal cortex relative to controls, which may indicate delayed neuronal pruning in this particular frontal region. The AR children also showed an interesting pattern of increased GM in temporal and parietal cortices which may indicate delayed neuronal pruning within the frontal, temporal, and parietal lobes in pre-pubescent at-risk children. Neurodevelopmental events that occur between the fifth and tenth years of life contribute to increased brain volume include cell growth, synaptogenesis, and arborazation (Pfefferbaum, et al., 1994). After this time, in normal childhood and adolescent brain development, GM has been shown to decline steadily, while WM volume and connectivity increases (Gogtay, et al., 2004).

An explanation into why frontal GM differences were not observed between the Sz and Con-A groups may be related to the symptomotology of the patients. Reduced GM in the PFC has been highly correlated with the presence of negative symptoms in schizophrenia patients (Koutsouleris, et al., 2008) and in those at-risk (Meisenzahl, et al., 2008). In our sample of Sz patients the presence of negative symptoms did not seem to be the most prevalent form of psychopathology. For example, the Sz patients had a substantially higher mean PANSS positive score of 25 (range 9-55) compared to their mean PANSS negative score of 19 (range 11-28). A reduction in prefrontal GM may be more indicative of those showing more negative symptomotology, whereas differences in temporal structures may be more pronounced in those with greater instances of positive symptoms.

The two regions, showing increased GM, which did not show a direct overlap in the conjunction analysis, but clearly fell in nearby cortical locations, were the left
angular and superior temporal gyri of the psychosis groups. Elevated activations have been observed in these two regions in an fMRI study of those experiencing auditory hallucinations (Zhang, et al., 2008). That study examined the relationship between auditory verbal hallucinations (AVH) with cerebral activity in schizophrenia patients who experience AVH and those who did not. They found that when external voices, which mimicked the AVH they experienced, were presented to the left ear the left angular gyrus increased activity only in the patients who regularly experience AVH, but not in those without that symptom. When the “voices” were presented to the right ear the left STG was more active. This suggests the positive symptom of AVH is associated with hyperactivity in the left lateralised language areas in patients. This would fall in line with the results of increased GM volumes in these regions in the two groups experiencing psychosis symptoms. Shapleske et al. (2001) found that greater left planum temporal GM correlated with higher positive symptoms score, particularly AVH symptoms. The PSY group did not reveal planum temporal activity during the functional task in this study, but this was expected as the response inhibition task administered was not intended to recruit language functions of the temporal lobe (Garavan, et al., 1999).

One region hypothesised to show GM reductions in the AR group was the cingulate gyrus (Pantelis, Yücel, Wood, McGorry, & Velakoulis, 2003). We found a significant GM reduction in the cingulate gyrus in the posterior region in the Sz patients, but not in the AR children. Previous similar findings to those found in the present study were observed in a study of young adults at-risk for psychosis who subsequently went on to develop the illness (Borgwardt, et al., 2007). They found reductions in GM in the posterior cingulate and insula, and increases in the posterior parietal/temporal lobe. Therefore, one possible interpretation from our results is that
the increased volume in the angular gyrus, located in the posterior parietal region of the brain, and the decrease in insular volume, which were all found in both the AR and Sz groups, may reveal vulnerability regions which are being detectable before the onset of the illness and potentially carrying through to the transition to the illness. While on the other hand the reduction in posterior cingulate volume found only in the Sz patients may occur closer to the onset of the illness.

**Conjunction**

The right anterior insula was the only region that showed a group difference between the PSY and CON groups. Previous studies have reported the insula to have multiple and varied functions, such as playing a key role in the decision to respond or inhibit by analyzing affective information (Shafritz, Collins, & Blumberg, 2006), assessing emotional valence of affective stimuli (Kotz, Meyer, & Paulmann, 2006), and subjective awareness of bodily states (Craig, 2009). Cytoarchitecturally, the volumetric difference observed in the current study lies within the anterior insula, which is a rostral ventral subregion of the insula. This paralimbic subcortical region is composed of agranular layers of neurons and has reciprocal connections with the amygdala and hippocampus via the ethorinal cortex (Amaral & Price, 1984). Carmichael and Price (1996) have posited that the insula integrates sensory and amygdaloid inputs. Neuroimaging research in macaque monkeys has revealed that the insula sends strong afferent projections to the ventral striatum, in the nucleus accumbens (NAcc), which helps to integrate feeding behaviours with rewards and memory (Fudge, Breitbart, Danish, & Pannoni, 2005). This coupling of visceral and cognitive processes has also been observed in addiction research. One study found that the insular cortex stores sensory representations of the enjoyable effects of drug use and those representations are recalled in the presences of cues associated with
taking the drug (Naqvi, Rudrauf, Damasio, & Bechara, 2007). Greater GM volume in
the anterior insula has been linked to greater accuracy during a subjective sense of
awareness of inner bodily states tasks (Critchley, Wiens, Rotshtein, Ohman, & Dolan,
2004). Allman and colleagues (2002) have discovered that the right insula contains
spindle neurons, also found in the ACC, which are specialised to regulate cognitive-
emotional processes and bring about self-awareness brought on by bodily and
emotional states. The right insula has also been hypothesised to play a role central in
the somatic marker theory (Damasio, 1996), which posits that conscious decisions
arises through insular interactions with the PFC.

Reductions in insular volumes have been previously reported in schizophrenia
patients at different stages of the illness (Honea, Crow, Passingham, & Mackay, 2005;
Shapleske, Rossell, Simmons, David, & Woodruff, 2001; Sigmundsson, et al., 2001;
GM in adults at genetic risk for psychosis. They found a cluster within the right
insular lobe showing reduced volume in the at-risk group compared to a healthy
control group. Kasai and colleagues (2003) found that the reduction in insular GM
seems to be specific to psychotic symptomatology. They found a reduction in bilateral
insula in first-episode psychosis patients, but not in patients with affective disorders.
Takahashi et al. (2009) conducted a longitudinal comparison of the insular cortex GM
changes in 31 UHR individuals (20 who subsequently developed psychosis and 11
who did not convert) and 20 controls whom had follow-up MRI data between one and
four years later. They found the at-risk subjects who developed psychosis had a
significantly reduced right insular GM volume compared with those at-risk who did
not convert and were healthy controls. More severe negative symptoms in the
participants who converted to psychosis were correlated with smaller volumes of the
right long insular cortex. Their study suggested that reductions in insular cortex GM may reflect pre-existing vulnerability for psychosis, and is also a region of neurodegenerative progressive during the transition period into psychosis. A study of the neurobiological underpinnings the risk for psychosis found that reduced glutamate levels in the thalamus, a region known to be heavily inter-connected with the insula, were correlated with reductions in GM volume in the insula in those at-risk (Stone, et al., 2009). Borgwardt and colleagues (2007) conducted a study of 35 ARMS individuals, of which 12 converted to psychosis. Whole-brain VBM found a reduction in the right insula in those who converted compared to those who did not. Reduced glutamate functioning was found in the thalamus, in individuals at-risk. This may be an underlying neurobiological factor that influences the volumetric difference in the insula found in both of psychosis groups.

In the single studies and combined group analysis for STOPS the insula did not show significant functional group activation differences during correct inhibitions. However, in the ABD study (Jacobson, et al., 2010; see Chapter Two) when an ERROR (i.e. incorrect inhibition) was made, the AR group showed a reduction in insular activity. Therefore, the activation in this region may reflect the emotion felt when the participant becomes aware that they have made a mistake. The recruitment of the insula has been shown in other tasks of attention and inhibition (Kirino, Belger, Goldman-Rakic, & McCarthy, 2000), indicating that the insula may become activated when the increasing demands of the inhibition task become greater than one’s ability to sustain their attention.

It would appear that in the maturing brains of the children at-risk that volumetric increase in temporal and parietal regions are early indicators of aberrant GM pruning that do not resolve in the adults diagnosed with schizophrenia. More
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interestingly, the reduced insular volume found in the PSY group, which is a region that has been found in studies of prodromal individuals who later convert to psychosis, maybe the earliest volumetric indicator of risk in the brain. This region has shown to play a central role in emotion regulation and is also highly interconnected to the PFC and the thalamus. A structural reduction in this region of the cortex could cause widespread disruptions during the course of development. If the structural reduction does not resolve during adolescent brain maturation this could cause a disruption in the ability to use top-down control between the PFC and insula to modulate affective experiences during new challenging adolescent social experiences. The previous study which mentioned greater negative symptomatology associated with decreased insular GM may also be a structural intermediate phenotype of the behavioural expression of an overall reduction in functioning, which is one of the core symptoms of the prodromal phase of psychosis.

3.5.3 DTI

Separate studies

There was extensive between-group differences in FA found between groups in the ChSz and ABD studies, in contrast to the limited findings of gross anatomical changes in the VBM analyses. Disruptions in WM integrity are evidenced by work exploring the functional disconnectivity hypothesis. This theory has proposed that disrupted communication between brain regions is based on altered synaptic connection strength rather than on structural macro-anatomical changes (Friston, 1998). There were regions of abnormal FA reductions observed in the adult Sz patients, mainly in the bilateral inferior frontal lobe along the uncinate fasciculus and superior longitudinal fasciculus, which were not found in the AR children. Therefore, differences found only in the adult Sz patients would be consistent with the theory
that suggest that the onset of schizophrenia coincides with aberrant synaptic pruning in the frontal lobes during late adolescence (McGlashan & Hoffman, 2000). However, both the AR and Sz patients revealed one common WM fibre tract of showing lower FA in both groups, the left cingulum.

**Conjunction**

Longitudinal MRI studies of age-related GM and WM changes have revealed that WM shows a linear increase with age, whereas GM volumes in frontal, parietal, and temporal regions reach peak levels during adolescence and decline with age, creating a reduction in the WM-to-GM ratio with increasing age (Bartzokis, et al., 2001; Giedd, et al., 1999; Sowell, et al., 2003). However, schizophrenia patients do not seem to reveal this normal pattern of WM and GM development. Instead, patients fail to increase WM with age, therefore a GM deficit is more apparent in younger patients and WM deficits become larger with age (Bartzokis, et al., 2001). Karlsgodt et al. (2008a, p.1300) have described neurodevelopmental pruning as a highly regulated process of axonal degeneration, which is mechanistically separate from either axonal retraction or from lesion-induced degeneration. The processes that initiate it may include changes in levels and distribution of excitatory and inhibitory neurotransmitters, and changes in the availability of neurotropic factors. Feinberg (1982) proposed that the onset of schizophrenia occurs when the programmed axonal pruning process and subsequent formation of new pathways, that normally take place during adolescence, is disturbed. The conjunction analysis set out to see what microstructural differences were shared in the AR and Sz groups relative to matched controls, potentially identifying abnormal WM regions which may be the result of have incurred aberrant neuronal pruning.
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The conjunction analysis revealed that FA reductions in WM are detectable in both the children at-risk and adult patients and occurred along specific fibre tracts within bilateral parahippocampi, bilateral cingulate, right nucleus accumbens, left fornix, right inferior parietal lobule, left precentral gyrus, and right postcentral gyrus. There were also increases in the PSY group within the right putamen, pons, left precentral gyrus, and right cingulate gyrus.

**Cingulum bundle**

The reductions in FA along the cingulum WM fibre bundle in the PSY group fell within bilateral parahippocampi and left posterior cingulate. The cingulum bundle tracts carry afferent connections from the cingulate gyrus to the PFC forming connections between the precuneus, hippocampus, and parahippocampus (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005), which would indicate its importance in co-ordinated inter-regional cortical functioning. An aberration within parahippocampal connectivity could lead to memory difficulties, since this region is thought to be a crucial substrate for memory acquisition, consolidation, and retrieval (Fell, Klaver, Elger, & Fernández, 2002). Volumetric reductions within the parahippocampus have been observed in schizophrenia patients (Bogerts, Meertz, & Schönfeldt-Bausch, 1985). The role of the parahippocampus in memory research has been observed in a PET study which found that the parahippocampus is recruited during the retrieval phase of visual spatial working memory tasks (Owen, Milner, Petrides, & Evans, 1996). Research into the neuropathology of positive symptoms in schizophrenia patients has revealed that the left parahippocampus is involved in the auditory verbal hallucination related activity in patients (Copolov, et al., 2003; Shergill, et al., 2004). DeLisi and colleagues (2006) utilised diffusion weighted imaging to investigate the appearance of cortical atrophy in individuals at a high
genetic risk (aged 12-30) along with schizophrenia patients (aged 20-55) and controls. They found an increase in the amount of CSF occupying the interstitial brain space in the region of the left parahippocampal gyrus in both patients and risk subjects. This increase indicates that tissue atrophy may be occurring in this region.

As closer examination of FA values in the PSY group, the reduction in FA in the left posterior cingulate seemed to be driven by the Sz group, whereas the AR group had comparable FA values to the control groups. This finding falls in line with the reduction in functional activity and GM volume found in the Sz patients, but not in the AR children. Previous studies reported FA reductions in the posterior cingulate in schizophrenia patients (Fujiwara, et al., 2007) have also been correlated with poorer attention and working memory performance (Kubicki, et al., 2003). A study of young adults at genetic-risk for psychosis found FA reductions within the left posterior cingulate gyrus, compared to healthy controls (Hoptman, et al., 2008). Therefore, the functional and structural reductions in the Sz patients may be genetically mediated and do not appear in a small sample of at-risk children. Furthermore, the reductions in the posterior cingulate may occur later in the course of the illness and maturational development.

**Superior longitudinal fasciculus (SLF)**

Both CON and PSY groups showed FA reductions within the left precentral gyrus along the SLF, therefore abnormalities in this motor cortex region do not seem to be specific to the presence of psychotic-like symptoms. However, FA reductions were observed in the PSY group within the right inferior parietal lobule at the terminus of the SLF. The SLF is a fibre tract of particular interest in schizophrenia, serving as the primary connection between frontal and parietal lobes and their role in supporting working memory functions (Petrides & Pandya, 2002). FA reductions
along the SLF have been found in schizophrenia patients (Szeszko, et al., 2008) and at baseline examination in individuals at a high genetic risk (Karlsgodt, Niendam, Beaden, & Cannon, 2009). Additionally, these reductions have been correlated with verbal working memory performance in first-episode, young adult patients (Karlsgodt, et al., 2008b). Maturational patterns between the frontal and parietal lobe regions have also been correlated with the patterns of maturation in increased working memory performance and functional activations (Klingberg, O'Sullivan, & Roland, 1997).

Normal adolescent maturational WM patterns reveal an age-related increase in FA along the SLF (Karlsgodt, Niendam, Beaden, & Cannon, 2009). Lastly, FA reductions in individuals at a high genetic risk for psychosis have been found within the right parietal lobe (Hoptman, et al., 2008). These results suggest that the reduction in the parietal region of the SLF observed in the PSY group may subserve impairments in working memory, that has been found to be a core deficit in schizophrenia (Lee & Park, 2005). Additionally, the PSY group revealed left lateralised hyperactivity in the precuneus during the functional task which may be indicative of a compensatory strategy used by the PSY group in the absence of optimal right hemispheric SLF microstructural integrity.

**Anterior thalamic radiation**

A reduction in FA in the PSY group was observed along the anterior thalamic radiation near the right NAcc. The mesolimbic dopamine pathway, which includes the NAcc, hippocampus, amygdala, and ventral tegmental area (VTA) has been associated with schizophrenia due to its role in emotional processing, learning, and reward salience attribution (Laviolette, 2007). The NAcc located in the ventral striatum receives its DA projections from the VTA, and has been associated with reward learning and motivation (Berridge, 2007). O'Doherty and colleagues (2004)
have attempted to disentangle the roles of reward learning in the ventral and dorsal striatum using fMRI. They conceded that the former serves as a "critic" that has the primary role of reward prediction and anticipation whereas the latter is the "actor" who is involved in the modulation of the stimulus-response reward association. Salience attribution, due to increased striatal DA has been shown to be aberrant in schizophrenia patients (Kapur, Mizrahi, & Li, 2005). However, the ventral striatum does not seem to be affected by an excess of pre-synaptic DA, instead, increased amounts of DA have been found in the dorsal striatum in both at-risk and schizophrenia patients (Fusar-Poli, et al., 2009b; Howes, et al., 2009). This would indicate that the reduced WM integrity in the PSY group in the ventral striatum may not be related to a DA dysfunction, but may have another underlying neurobiological cause driving this micro-structural difference.

**Commissure of the fornix**

The fornix is a compact bundle of WM fibres which connects the hippocampus of each cerebral hemisphere, which is located in the medial temporal lobe (Snell, 2006). DTI studies have revealed that the hippocampus connects to the thalamus and PFC (Kubicki, et al., 2005). Kuroki and colleagues (2006a) found a reduction in FA along the fornix and volumetric reduction in the hippocampus in schizophrenia patients. The hippocampus has long been believed to play a central role in memory formation since the seminal studies of H.M. (Scoville & Milner, 1957). WM FA reductions in the hippocampus have been found in schizophrenia patients (White, et al., 2007). A cross-sectional study of FA in a high-risk population has revealed that during normal adolescent development, healthy controls increase FA with age in the hippocampus and temporal lobes, whereas the high-risk subjects failed to show that same increase (Karlsgodt, Niendam, Beaden, & Cannon, 2009). A
disruption in inter-hemispheric connectivity and synaptic organisation between the two hippocampi could result in the disturbances in memory formation frequently found in schizophrenia patients (Boyer, Phillips, Rousseau, & Ilivitsky, 2007). A substantial amount of volumetric studies have reported reductions in hippocampal volume in both medicated and un-medicated schizophrenic patients (Harrison, 2004; Shenton, Duckey, Frumin, & McCarley, 2001; Wright, et al., 2000). Harrison and colleagues (2004) have suggested that the volumetric changes probably arise from altered development involving aberrant synaptic organisation and connectivity within the WM.

**External capsule**

Within the external capsule an increase in FA was found in the PSY group within the right putamen, a component of the dorsal striatum. Functionally, both the AR and Sz groups revealed hypoactivity in the dorsal striatum. This region of the brain receives dopaminergic innervations via the mesolimbic DA system. Efferent excitatory cortical projections to the putamen are glutamatergic in nature and are balanced by the inhibitory dopaminergic input to the putamen from the substantia nigra. Excessive DA in the mesolimbic and mesocortical dopaminergic systems has been suggested to cause hypertrophy of connections in individuals at high risk (Hoptman, et al., 2008). Higher levels of DA have been found in the putamen and caudate in the unaffected siblings of schizophrenia patients (Huttunen, et al., 2008) suggesting a genetic liability of this region of the vulnerability for psychosis. Increased FA has also been previously reported in the putamen during childhood brain development (Snook, Paulson, Roy, Phillips, & Beaulieu, 2005).

Hoptman and colleagues (2004) found WM abnormalities in the caudate which correlated with impulsivity measures in schizophrenia. They also found that
better GO/Nogo task performance was negatively correlated with a reduction in FA in the caudate. Casey and colleagues (1997) found disrupted fronto-striatal circuitry in children with attention deficit hyperactivity disorder (ADHD) during a response inhibition task. Their data suggest that the role of the right PFC is in suppressing responses to salient, but otherwise irrelevant events, while the basal ganglia appeared to be involved in executing the behavioural response.

The dopamine hypothesis of schizophrenia posits that schizophrenia patients experience hypodopamanergia in the PFC and compensatory hyperdopamanergia in the striatum. Davis et al. (1991) has theorised that the negative symptoms in schizophrenia results from reductions in dopamine levels in the PFC, whereas positive symptoms results from the striatal increases in DA. Abnormally high DA levels in midbrain regions have been strongly linked not only to those diagnosed with schizophrenia, but also in individuals in the prodromal phase of the illness (McGuire, Howes, Stone, & Fusar-Poli, 2008) and genetically high-risk individuals, who show an increases in pre-synaptic DA in the dorsal striatum (Howes, et al., 2007). Localization of pre-synaptic DA was examined in a PET study of ARMS and first-episode patients (Mean age: 26 years old) which found that both groups had elevated DA levels in the dorsal striatum (an association region), but not in the ventral striatum (limbic region) or posterior striatum (sensorimotor regulation), relative to a control group (Howes, et al., 2009). Therefore, the increased FA in the dorsal striatum observed in the children and adult psychosis groups may reflect the excess in dopamanergic tone in the putamen resulting in hypertrophy in surrounding WM fibres which may lead to the dysfunctional programmed axonal pruning, as posited by Feinberg (1982), causing disruptions to normal developmental circuitry. Or on the other hand, this increase may represent excessive myelination which can persist into
adulthood. However, the only way to parse out the underlying causal mechanism would be to follow this FA increase through adolescence into adulthood in the at-risk group to see if the onset of puberty affects this finding. Future multi-modal work, with MRI and PET, will need to be carried out in individuals at-risk to examine the role of dorsal striatal DA release in relation to functional activations during tasks of response inhibition.

**Middle cerebellar peduncle**

Increased FA was found in the PSY group within the pons which connects to the cerebellum via the middle cerebellar peduncle. This region is located in the brainstem and is a central relay system between the cortex, in particular the putamen, and the cerebellum (Snell, 2006). Afferent fibres from the cortex climb down to synapse in the pons, neurons then enter the cerebellum along excitatory mossy fibres (Snell, 2006). An FA increase within the dorsal pontine tegmentum, which is the region of synapse in the pons before neurons enter the cerebellum, has been found in individuals with a high genetic risk for psychosis compared to healthy controls (Hoptman, et al., 2008). In a study which administered L-dopa, a dopamine precursor, during resting-state scans they observed greater connectivity between the putamen and cerebellum in the L-Dopa condition versus a placebo condition (Kelly, et al., 2009b). Anisotropy increases in this region may be an indicator of greater cortico-cerebellar-thalamo-cortical (CCTCC) connectivity within the PSY group. Andreasen et al. (1996; 1998; 1999) has suggested that the disorganised symptoms of schizophrenia, known as “cognitive dysmetria,” arise from disruptions within the CCTCC circuitry in the brain, which is a network that subserves the coordination of both motor and cognitive processes (Middleton & Strick, 2000; Schmahmann, 1991). This connectivity path could be compensatory in response to the reductions in fronto-
parietal and fronto-limbic connectivity, which showed FA reductions in the PSY group. This hypothesis is supported by the trend for increased right cerebellar tonsil activity found in the PSY>CON comparison [$t(53)=-1.83, p=.07$] during the response inhibition task.

Recent literature describing prodromal populations has begun to focus on the medial temporal lobe as a core region of WM disconnectivity, and may play a pivotal role in working memory dysfunctions in schizophrenia. The current findings of reduced WM integrity in the PSY group within bilateral parahippocampus echo findings of a prodromal sample who also had a deterioration in social and role functioning over a longitudinal course (Karlsgodt, Niendam, Bearden, & Cannon, 2009). This micro-structural alteration in the children at-risk may be caused by disrupted developmental mechanisms, most likely decreased myelination in this region, which could not resolve in adulthood and potentially lead to the severe working memory impairments observed in patients with schizophrenia.

### 3.6 Conclusions

In this chapter I set out to systematically compare brain activity, morphometry and WM integrity between an at-risk group of children with subclinical psychotic symptoms and chronic schizophrenia patients compared to healthy matched controls. Functional results revealed a triad of hypoactivity within the prefrontal, temporal, and claustrum regions in the PSY group. Previous findings of right PFC hypoactivation indicate that a dysfunction in this region is central to the cognitive deficits found in schizophrenia (Hill, et al., 2004). I observed this PFC hypoactivity in both the AR children and those with the established illness. The hypoactivity in the right IFG and SFG regions could potentially be the origins of the cascade of hypoactivity in the brain in the PSY group which may carry into the middle temporal lobe and claustrum.
Both of these regions are known to be important in integrating semantic, linguistic, and sensory information (Calvert, 2001; Ettlinger & Wilson, 1990). The PSY group appear to rely on a compensatory strategy of recruiting the less efficient precuneus in the posterior brain region to successfully perform the cognitive task. This pattern of activity validates the inefficiency hypothesis, which posits that hyperactivity is a result of inefficient and less automatic processing within the PFC when the participant is performing well on a task and is evidenced by extraneous neural activity (Weinberger & McClure, 2002). Ramsey and colleagues (2002) have suggested that the inefficiency of neural communication observed in schizophrenia patients results in excessive recruitment of neural systems to perform at comparable levels to controls during cognitive tasks. The recruitment of posterior regions can also be observed in the developmentally immature brains of children who show more diffuse, less active and widespread patterns of activation compared to adults during working memory tasks (Braet, et al., 2009; Rubia, et al., 2000; Thomas, et al., 1999). Additionally, the recruitment of posterior regions of the brain that are less efficient has been observed in a study of otherwise healthy individuals at-risk for psychosis who experience psychotic symptoms (Whalley, et al., 2004).

My results validate previous theories of schizophrenia that highlight the role of altered brain connectivity which manifest at the macro-structural and functional level, as a result of micro-structural changes in WM tracts. The lack of frontal FA differences observed between the adults and children of the PSY and CON groups, but found in the reduced frontal FA in adult Sz patients only, likely reflects the immature cortical developmental stage in the AR children. However, reductions in expected activations in right frontal regions during the inhibition task were found in the PSY group, and may be a result of the abnormal micro-structural WM found along
the PFC-connecting cingulum bundle in the PSY group. Additionally, the PSY group exhibited a reduction in FA within the right NAcc, in the ventral striatum with VTA inputs, and an increase in FA within the putamen, in the dorsal striatum with substantia nigra inputs, both of which receive dopaminergic innervation. Anatomical segregation of the functions of the striatum has deemed the ventral region of the striatum as the “critic” which is involved in reward prediction and motivation, whereas the dorsal region of the striatum as the “actor” which modulates the stimulus-response-reward association (O'Doherty, et al., 2004). Therefore, a deficit in the ventral striatum could lead to the dysfunctions in expected rewards during anticipation. Whereas hypertrophy, evidenced by increased FA, in the dorsal striatum could lead to a dysregulation in the release of DA that enters this mesolimbic pathway. The elevated DA in the dorsal striatum, which have been theorised to cause salience misattribution in schizophrenia (Kapur, et al., 2005), is believed to be a compensatory in response to depleted DA levels in the PFC. Aberrant salience attribution leads to an inability to monitor and filter relevant or irrelevant stimuli, which is a distinction that is needed to carry out appropriate goal-directed behaviours. I am currently collecting fMRI data from a separate study of individuals at-risk that experience psychotic-like symptoms, to directly assess ventral striatum functioning during a reward task. Lastly, with respect to functional connectivity patterns the fMRI and FA results points to greater cortico-striatal-cerebellar connectivity within the PSY group, which may arise due to deficits in frontal and temporal functioning. Alternatively, the CON group utilised a more efficient fronto-midline-temporal connectivity network.

Future neuroimaging studies in at-risk groups will need to fully explore the presence of the subclinical psychotic symptoms, in particular the most frequent symptom of auditory verbal hallucinations in the children at-risk. Our fMRI task was
not intended to recruit temporal language regions. Therefore, future research will need to use tasks mimicking the “voices” they hear, to see if our finding of increased GM in the temporal and angular regions is related to increased activity in the aforementioned regions. Future at-risk investigations should also include tasks which require the processing of emotional valence, to target insular and amygdala functioning, and address if the volumetric reduction in the insula could underlie blunted emotional states which have been observed in chronic patients. Additionally, future DTI studies with risk groups should have high enough spatial resolution (i.e. 60 directions or more) to allow for a tractography analysis, which would allow for a more accurate assessment of neuronal functional connectivity. In particular, one could isolate an abnormality in FA and follow the individual tract it falls on to see if any difference in functional activity lie along that tract. Additionally, with a higher number of diffusion sensitisation gradients one could more thoroughly differentiate the complexity of the organisation of WM tracts, to observe if changes in FA are truly related to WM integrity, or if they are related to crossing fibres.

Another potential confound is that all of the chronic schizophrenia patients were on neuroleptic treatment, whereas none of the at-risk children were on medication. This did not allow me to separate functional and structural differences seen in the patient group that may be attributed to neuroleptic effects. Antipsychotic medication has been shown to be effect functional activity during fMRI cognitive tasks (Mendrek, et al., 2004) and also grey and white matter in schizophrenia patients (Molina, et al., 2005). Future longitudinal studies of risk groups need to include a non-medicated first-episode patient control group along with medicated patients to parse out the effects of medication on the brain.
Due to the difficulty involved in the recruitment of the unique at-risk population and the limited access schizophrenia outpatients the small sample size of these studies were relatively small and may have not allowed for more subtle differences in brain structure and function to survive the stringent statistical thresholds employed in my analyses. It is also important to note that the at-risk group reported here contains an admixture of individuals whose symptoms may disappear over the course of adolescence and those who may go on to develop some form of psychosis, with vulnerability rates between 16- to 65-fold (Poulton, et al., 2000; van Os, Linscott, Myin-Germeys, Delespaul, & Krabbendam, 2009). Future work should concentrate on longitudinal assessments of pre-pubescent children who report subclinical psychotic symptoms and assess functional and structural measures at regular intervals between baseline and transition to the illness, therefore removing the false-negatives that may contaminate the results of the current study. A potential goal could then be to isolate regions in the brains of those who do not convert that might protect them against the conversion to the illness. These regions could serve as key regions to target in therapeutic interventions during the risk state. A comprehensive review of the early intervention treatment studies in ultra-high risk patients (de Koning et al., 2009) concluded that cognitive behavioural therapy and/or antipsychotic medication and/or family intervention and/or social skills training suggest optimistic methods of delaying psychosis onset by the end of treatment. However future longitudinal studies beginning in childhood through the late adolescent age risk window need to be carried out and tailored specifically to the current neurodevelopmental stage to determine if early intervention targeting a priori brain regions can prevent the conversion to the frank onset of schizophrenia.
4. Chapter Four
Resting-state functional connectivity reveals reduced intra-regional communication in children at-risk for psychosis
4.1 Abstract

This study investigated the neural substrates of resting-state functional connectivity in an at-risk cohort of children, from the Adolescent Brain Development study (ABD study) (Jacobson, et al., 2010; see Chapter Two), and patients with chronic schizophrenia from the Chronic Schizophrenia study (ChSz study) (see Chapter Three). A between-subjects assessment of brain functioning during resting-state fMRI took place among 11 children with subclinical psychotic symptoms who were at-risk (AR) for developing psychosis and 14 healthy control children (Con-C) and separately for 17 chronic schizophrenia patients (Sz) and 16 matched healthy adult controls (Con-A). Seed ROIs were derived from the ABD and ChSz studies in regions which revealed between-group differences during a response inhibition task. An additional seed region derived from the VBM analysis revealed a grey matter (GM) reduction in both the AR and Sz participants, relative to controls. The analysis revealed a pattern of reduced functional connectivity in the AR and Sz groups within and between numerous right hemisphere regions that encompassed frontal and parietal cortex and the striatum. The regions revealing reduced functional connectivity in the PSY groups also showed reduced activation during the response inhibition task. Our findings suggest that there are common patterns of aberrant functional organisation between AR and Sz groups during the resting state which may underlie the dysfunctional task-related activity, and these abnormalities are observable during the risk stage and continue after onset of the illness.
4.2 Introduction

4.2.1 Disconnection hypothesis of schizophrenia

The disconnection hypothesis in schizophrenia research is a widely accepted theory that states that when a focal disruption occurs the entire network will be adversely affected (Bullmore, Frangou, & Murray, 1997; Friston & Frith, 1995; Weinberger, Berman, Suddath, & Torrey, 1992). Friston (1998) posited that this functional disconnectivity, or disrupted communication between brain regions, is based on altered synaptic connection strength rather than on macro-anatomical changes. Empirical evidence for this model is drawn from fMRI studies revealing that deficits in cognitive and affective behaviours in schizophrenia may be attributed to a failed ability to integrate inter-regional, dispersed neural networks (Andreasen, et al., 1999; Benes, 2000; Selemon & Goldman-Rakic, 1999). Specific reductions in task-related functional connectivity in schizophrenia have been found between fronto-temporal regions (Johnstone, Lawrie, & Cosway, 2002; Stephan, Baldeweg, & Friston, 2006), cortico-cerebellar-thalamo-cortical circuitry (CCTCC) (Honey, et al., 2005), fronto-parietal regions (Kim, et al., 2003b), and temporo-anterior cingulate cortex (Boksman, et al., 2005).

4.2.2 RSFC

Recently attention has been drawn to fMRI studies reporting findings of intrinsic, correlated activity in the brain in the absence of a functional task (Raichle & Mintun, 2006). Biswal and colleagues (1995) were the first to report low-frequency fluctuations in spontaneous neural activations in the BOLD signal within the motor cortex when the participant was at rest and not performing a task. Resting-state functional connectivity (RSFC) is a relatively new technique which has been advancing the understanding of the complex relationships between distributed brain...
regions subserving cognition. Studies of RSFC examine the correlations in slow spontaneous fluctuations (<.1 Hz) in the BOLD signal, without the influence of task-related BOLD changes. Identifiable resting networks have shown consistency across individuals (Damoiseaux, et al., 2006). The recent work of Smith and colleagues (2009) identified the major activation networks that were engaged during resting-state (n= 36) during fMRI and PET scans, which were derived from nearly 30,000 human participants’ scans across 66 different behavioural task domains. They have established that the functional organisations of the resting networks are almost identical to those during behavioural tasks eliciting functional activations, especially within the visual and sensorimotor networks. During resting-state healthy controls have shown deactivations in regions that are engaged during cognitive tasks within the frontal and parietal cortices (Laufs, et al., 2003; van de Ven, Formisano, Prvulovic, Roeder, & Linden, 2004), which suggests that the brain regions that support executive functioning tasks are disengaged during the resting-state. In contrast, high frequency fluctuations during rest are positively correlated in retrosplenial, temporo-parietal, and dorsal medial prefrontal (dmPFC) cortices (Laufs, et al., 2003).

Two independent research groups have examined RSFC in healthy controls and found anti-correlations between two distinct networks (Fox, et al., 2005; Fransson, 2005). These networks were found through intrinsic correlations between commonly activated/deactivated regions and defined as the “task-positive” network, which is engaged in goal-directed task performance, and the “task-negative” network, which is commonly deactivated during cognitive tasks and more active during resting-state. Regions in the task-positive network include the DLPFC, VLPFC, insula, supplementary motor area, intraparietal sulcus and frontal eye field (Fox, et al., 2005). These regions are commonly associated with functions related to attention (Laufs, et
Task-negative network revealed common reductions in activity during cognitive tasks in the posterior and anterior cingulate, precuneus, angular gyrus, cerebellar tonsil, lateral parietal cortex, left medial PFC, left superior frontal gyrus, inferior temporal gyrus, and parahippocampus (Fox, et al., 2005; Mazoyer, et al., 2001). Regions in this network have been linked to cognitive processes that occur in stimulus-independent thought, such as self-referential tasks (Gusnard & Raichle, 2001).

4.2.3 RSFC in schizophrenia patients

The system of synchronised task-related deactivations, known as the task-negative network, which is suspended during goal-directed behaviours has been termed the “default-mode network” (DMN) of the brain (Raichle, et al., 2001). The DMN is conceptualised as a stimulus-independent network demonstrating low frequency oscillations that decrease during task-related activity and increase during self-monitoring and reflection (Gusnard & Raichle, 2001). Studies of the DMN in schizophrenia patients are limited but have revealed RSFC abnormalities. However, the studies reported are not consistent in their findings (see review in Greicius, 2008).

The first study of RSFC in schizophrenia (Liang, et al., 2006) revealed widespread differences in connectivity between patients and controls, which were mainly decreases (n=158) but also included increases (n=19) in connectivity between regions in patients. Bluhm and colleagues (2007) found schizophrenia patients had fewer correlations between low frequency activities in DMN regions, which included the posterior cingulate seed region and lateral parietal, medial PFC, and cerebellar regions, compared to controls. Zhou et al. (2008) collected RSFC and DTI in schizophrenia patients and a connectivity analysis was conducted between an anterior hippocampal seed ROI and the rest of the brain. In patients a reduction in bilateral
Resting-state functional connectivity in children at-risk for psychosis

hippocampal RSFC was found between DMN regions, which are also integral brain regions subserving episodic memory (posterior cingulate, medial PFC, and parahippocampus) (Goldman-Rakic, 1999); additionally, these regions all have connections to the DLPFC (Petrides & Pandya, 1984). They also found reduced FA in the WM of the body of the fornix, which is a bundle of WM fibres connecting the two hemispheres of hippocampi. Additionally, in a study of elderly patients with a mild cognitive impairment (Sorg, et al., 2007), individuals who were at an elevated risk for developing Alzheimer’s Disease, researchers found reduced hippocampal RSFC in DMN regions (posterior cingulate and medial PFC). These studies of hippocampal FC reveal a region that may potentially underlie the memory impairments observed in the disorganised symptoms exhibited in schizophrenia patients. Hoptman and colleagues (2009) examined a seed region within the amygdala and its relation to aggression in schizophrenia. A RSFC analysis revealed reduced amygdala connectivity with ventral PFC, ACC, insula, and lentiform nucleus. Amygdala connectivity was negatively correlated with levels of aggression in patients. An exploratory seed region in the orbitofrontal gyrus, a region associated with impulsivity (Bonelli & Cummings, 2007), revealed reduced RSFC within DMN regions (ACC, inferior parietal cortex, medial PFC), insula and claustrum. Therefore, these reductions in frontal-subcortical-parietal connectivity in the schizophrenia patients reveal an increased vulnerability to aggressive and impulsive behaviours. Correlations with negative symptoms have been investigated in schizophrenia through the use of PET during resting-state scans. A negative correlation was found between the increase in physical anhedonia scores and reductions in the DMN region of the medial PFC (Park, et al., 2009). Additionally, PANSS negative symptom scores were positively correlated with increased resting-state activity in the cerebellum. Therefore the comprised RSFC networks described in
patients could give rise to reduced integration in cortical synchrony across the entire brain (Bressler, 2005) and give rise to socially unacceptable behaviours. Cortical synchrony is when the firing of neurons in the brain are believed to be one of the mechanisms which allows related information to be bound together and processed in a coherent manner (Singer, 1994).

Conversely, independent component analyses (ICA) revealed findings of widespread increased in RSFC in the DMN of schizophrenic patients and also in frontal, parietal, occipital lobes, and basal ganglia connectivity. The only reductions in the patient’s RSFC were found in temporo-parietal connectivity (Jafri, Pearlson, Stevens, & Calhoun, 2008). Pomarol-Clotet and colleagues (2008) found that DLPFC activity was reduced in schizophrenia patients during a working memory task, and the DMN (medial PFC), was increased during the resting-state. These findings indicate both a failure to activate task-positive regions and deactivate task-negative regions. Although there was an overlap in patients from a previously reported group (Liang, et al., 2006) which revealed RSFC reductions, Zhou and colleagues (2007b) reported increased correlations of the RSFC in patients in both the DMN (medial PFC, posterior cingulate, precuneus, left parietal cortex, and inferior temporal gyrus) and in the task-positive networks (right DLPFC, supplementary motor area, and bilateral orbitofrontal gyrus). Since both of these networks are co-activated during rest a possible interpretation of these results could be that increased connectivity between both task-positive and negative networks creates excessive competition for brain resources. Therefore, I would hypothesise that patients become overwhelmed with both endogenous and exogenous stimuli, which interfere with the ability to carry out both goal-directed behaviours and monitor internal states.
There have only been two reports of RSFC in first-episode patients to date (Lui, et al., 2009; Zhou, et al., 2007a). Zhou and colleagues (2007a) used a seed region approach to investigate RSFC in medicated first-episode patients. The task-positive DLPFC seed was found to show reduced negative correlation in the patients between the posterior cingulate, parietal cortex, thalamus and striatum. Conversely the DLPFC seed had increased connectivity with left middle temporal and paralimbic regions. Lui and colleagues (2009) used seed ROIs derived from their findings of GM reductions in three cortical regions in antipsychotic-naïve first-episode schizophrenia patients and correlated RSFC in these regions with symptom scale scores. Increased RSFC in the middle temporal gyrus seed and putamen demonstrated a positive correlation with PANSS thought disturbance scores. However, a decrease between connectivity of DMN regions of the middle temporal gyrus seed and precuneus revealed a positive correlation with negative symptom scores, especially anergia, which is displaying a lack of energy. Negative symptomology was found to be related to greater temporo-striatal connectivity and reduced temporo-parietal connectivity. Reductions in temporo-parietal connectivity in the un-medicated patients resonate the findings of Jafri and colleagues (2008) in the chronic patients.

In summary, consistent evidence of RSFC abnormalities in schizophrenia have to be yet to be defined. The DMN has been the focus of most studies of connectivity in schizophrenia, due to the interest in schizophrenia research to determine the neurobiological mechanism, which may disrupt the ability to filter out irrelevant stimuli. Increased DMN activity in schizophrenia has been theorised to results in a competition between neuronal circuits. This contest between available neural networks during goal-directed behaviours may be a result of a failure to efficiently coordinate brain networks.
At present there have not been any investigations of RSFC in the risk for psychosis. The current study employed a symptomatic-risk approach of studying children at-risk for psychosis (see Chapter Two). Longitudinal research has shown that children who report subclinical psychotic symptoms are at significantly increased risk, ranging from 16- to 65-fold increase, of developing a clinical psychotic disorder (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005; Poulton, et al., 2000; van Os, Linscott, Myin-Germeys, Delespaul, & Krabbendam, 2009). Poulton and colleagues (2000) conducted a birth cohort study in New Zealand and found 11-year olds with self-reported psychotic symptoms were at a 16-fold increased risk for an adult psychotic illness.

I previously reported a cross-sectional design between children at-risk and adult schizophrenia patients to assess brain structure and function related to the established illness and the risk for developing schizophrenia. Two separate studies were conducted and included the ChSz study (see Chapter Three), of chronically ill schizophrenia patients and healthy adult controls; and the ABD study (Jacobson, et al., 2010; see Chapter Two), that included children who were experiencing subclinical psychotic symptoms and asymptomatic healthy control children. The schizophrenia patients revealed reduced activity in the right inferior frontal gyrus (IFG) and also in bilateral caudate and hypothalamus. Patients showed hyperactivity within the left superior frontal gyrus (SFG). The at-risk children showed reduced activity in areas that were predominantly right lateralised and included the IFG, precuneus, lentiform nucleus, middle temporal gyrus, left middle temporal gyrus and left claustrum (see Chapter Two, Table 2.2 and Figure 2.4). There were four regions with hyperactivity in the AR group, which were left lateralised and included the left medial frontal gyrus (BA32), left rostral ACC, left SFG, and left precentral gyrus (see Chapter Two, Table
2.2 and Figure 2.4). I applied a combined group analysis that identified regions which were activated in the schizophrenia patients (Sz) and at-risk children (AR), the combined PSY group, and also in the control adults (Con-A) and children (Con-C), the combined CON group. This analysis revealed that the PSY group showed hypoactivity within the right IFG, SFG, left middle temporal gyrus, and left claustrum. The PSY group showed increased activity in one region, left precuneus, which was driven by the AR group (see Chapter Three, Table 3.7). Together these functional results revealed a triad of hypoactivity within the prefrontal, temporal, and claustrum regions for the PSY group. These results suggest that the hypoactivity in the right frontal regions, IFG and SFG, may lead to the reduced activity in other regions found in the PSY group in the middle temporal lobe and claustrum, which are regions known to be important in integrating semantic, linguistic, and sensory information (Calvert, 2001; Ettlinger & Wilson, 1990). Previous findings of reduced right PFC activation indicate a dysfunction in this region is central to the cognitive deficits found in schizophrenia (Hill, et al., 2004).

Chapter Three demonstrated functional activity reductions in the AR group in the right PFC, temporal lobe, and claustrum for the AR group, but there was only one region in the inferior temporal lobe which revealed a reduction in GM volume. The Sz patients revealed similar reductions in functional activity as the AR children, but revealed reduced GM in the posterior cingulate and occipital lobe. However, a conjunction analysis of the VBM data between the PSY and CON groups detected one area of reduced GM in both the AR and Sz groups compared to controls, which fell within the right anterior insula. This reduction could reveal a potential locus of the affective dysfunctions observed in schizophrenia.
In this report I set out to find resting-state functional connectivity networks in regions of functional interest between the at-risk children with subclinical psychotic symptoms and the chronic schizophrenia patients compared to healthy matched controls. To investigate patterns of functional connectivity seed regions were chosen based on either between-group differences that were task-related or the conjunction GM volume-related. The aims set forth in this report were to examine the relationship of fronto-temporal, fronto-parietal, and fronto-cerebellar connectivity stemming from the seed ROIs. I hypothesise that those diagnosed with schizophrenia will show reduced resting-state connectivity in fronto-cingulate and fronto-parietal networks, which will also be observed, potentially to a lesser extent, in the children at-risk.

4.3 Materials and methods

4.3.1 Participants

Chronic schizophrenia study

The recruitment and demographics of the schizophrenia patients and controls in the ChSz study have been previously reported in Chapter Three. Following a complete description of the study, all 33 participants gave written consent to participate. The resting-state sample consisted of 17 participants with schizophrenia (Sz) (age range: 26-61) and 16 healthy adult controls (Con-A) (age range: 25-58) (see Chapter Three, Table 3.1).

Adolescent Brain Development (ABD) study

The details of the identification of the participant sample for the ABD study can be found in Chapter Two and additionally in Jacobson et al. (2010). The resting-state sample consisted of 25 children, 14 control children (Con-C), and 11 children with subclinical psychotic symptoms (AR) (see Chapter Three, Table 3.2).
4.3.2 fMRI task

The functional task which produced the seed regions has been previously described in Chapters Three and Four. The task was a motor GO/NOGO task which assessed the ability to inhibit a pre-potent response during functional imaging.

4.3.3 Imaging acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a 30.5 cm internal diameter three-axis local gradient coil with insertable radiofrequency coils with transmit–receive capabilities. Participants viewed stimuli back-projected onto a 640 x 480 LCD screen at the head of the scanner bed viewed through a mirror affixed to the head coil. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV = 230 mm, voxel size: 0.9 mm × 0.9 mm × 0.9 mm) were acquired. Task and resting-state functional data were collected using a T2* weighted echo planar imaging (EPI) sequence that acquired 32 non-contiguous (10% gap) 3.5 mm axial slices covering the entire brain (TE = 35ms, TR = 2000ms, FOV = 224 mm, 64 mm × 64 mm matrix size). At the beginning of the functional and resting-state scan four discarded acquisition scans (8s) were taken to allow for the stabilisation of T1 effects on the BOLD signal. Three minutes and 30 seconds of resting-state data was acquired from the children. Six minutes and 50 seconds of resting-state data was acquired from the adults. To facilitate analysis, this was truncated match the length of the children’s resting-state data.

4.3.4 fMRI analysis

The fMRI analyses have been previously described in Chapters Two and Three. Functional analyses were conducted using AFNI (Cox, 1996).
4.3.5 Analysis of the resting-state data

The method employed in this analysis is similar to that described elsewhere (Kelly, et al., 2009a). Figure 4.1 shows the processing pipeline.

Figure 4.1 Resting-state data analysis pathway for pre-processing, extraction of region of interest and nuisance convariate timeseries, individual subject multiple regression analyses, and mixed effects analyses of final group results. Adapted from a personal correspondance with a study collaborator, Kelly et al. (2009a).
4.3.6 Pre-processing

Initial pre-processing was performed using AFNI (Cox, 1996). This consisted of slice-time correction, and motion correction by aligning to a base image as described above. Most subsequent pre-processing was performed using FSL (Smith, et al., 2004). These steps consisted of spatial smoothing using a Gaussian kernel (5 mm FWHM), global mean-based intensity normalisation (each participant’s 4-D file was scaled by its mean), and temporal bandpass filtering ($0.005<f<0.1$) using AFNI’s 3dFourier. FSL’s FLIRT (Jenkinson, Bannister, Brady, & Smith, 2002; Jenkinson & Smith, 2001) was used to register the high-resolution T1 images to the 2 mm x 2 mm x 2 mm MNI152 template (Montreal Neurological Institute). FLIRT was also used to register each participant’s functional time-series to their high-resolution anatomical images.

4.3.7 Nuisance signal regression

Several sources of spurious variance were removed, using regression, from the data to control for physiological fluctuations occurring during respiratory and cardiac cycles (Fox, Snyder, Zacks, & Raichle, 2006). The nuisance regressors included (1) the six motion estimates from motion correction, (2) whole-brain signal averaged across all voxels in the brain, (3) signal from WM, and (4) signal from CSF. Correction for auto-correlation within the time-series was performed using FSL’s FILM. The WM and CSF regressors were produced by automatically segmenting a participant’s structural images using FAST. The segmented images were thresholded to ensure a 90% tissue probability. The resulting images were used to mask each participant’s time-series and an average time-series was extracted from all voxels within the mask.
The result of regressing out the auto-correlations in the nuisance signals created pre-whitened 4-D residuals, which had underwent correction for auto-correlation within the time-series, for each participant. To ensure that the functional connectivity estimates represent actual correlations, rather than regression coefficients, these 4-D files were scaled by dividing each time-series by its standard deviation. Furthermore, this step also removes potential between-group differences in the magnitude of the BOLD signal (Sorg, et al., 2007). Finally, the scaled residuals were transformed into MNI152 standard space at 2 mm x 2 mm x 2 mm resolution. This was accomplished by using FLIRT to apply the previously computed affine transformation that aligned each participant’s time-series to their high-resolution structural images.

4.3.8 Seed selection and creation

The centre of mass (COM) from the clusters identified for STOPS in the response inhibition task for the ChSz study, ABD study and combined group analysis between the two psychosis and two control groups were used as the seeds for the functional connectivity analyses (Table 4.1). One additional seed region derived from the VBM conjunction analysis was also used. The COMs were derived from Talairach and Tournoux (1988) co-ordinates from the functional analyses into MNI coordinates, to facilitate the RSFC analysis in FSL. Around each of the MNI-transformed COMs a sphere of four mm radius was drawn at 2 mm x 2 mm x 2mm resolution, which included 33 voxels occupying 264 mm x 264 mm x 264 mm. A total of 22 seed ROIs were examined. These ROIs were then used as masks and an average time-series, averaged over all voxels in the seed for each ROI and for each participant, was extracted from the pre-whitened residuals. The extracted time-series were then scaled by their standard deviations.
Table 4.1
Seed regions for the resting-state functional connectivity analysis.
These clusters were derived from whole-brain, voxelwise analysis (p<.05, corrected). These results are from the post-hoc ANOVA (p<.05) of the brain regions revealing between group differences during successful inhibitions (STOPS) among the ChSz study groups, the ABD study groups, and the combined group analysis of the two studies datasets.

<table>
<thead>
<tr>
<th>Seed region</th>
<th>Side</th>
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<th>L</th>
<th>P</th>
<th>I</th>
<th>Direction</th>
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<tbody>
<tr>
<td><strong>ChSz study – STOPS</strong></td>
<td></td>
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<tr>
<td>Posterior cingulate gyrus**</td>
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<td>31</td>
<td>2</td>
<td>-40</td>
<td>35</td>
<td>Con-A&gt;Sz</td>
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<td>R</td>
<td>46</td>
<td>55</td>
<td>31</td>
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<td>Con-A&gt;Sz</td>
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<td>-42</td>
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<tr>
<td>Caudate*</td>
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<td>20</td>
<td>-13</td>
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<td>Con-A&gt;Sz</td>
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<tr>
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<td>Inferior frontal gyrus*</td>
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<td>Con-C&gt;AR</td>
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<td>-47</td>
<td>-52</td>
<td>1</td>
<td>Con-C&gt;AR</td>
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<tr>
<td>Claustrum**</td>
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<tr>
<td>Superior frontal gyrus***</td>
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<td>22</td>
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<td>CON&gt;PSY</td>
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<tr>
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<tr>
<td>Insula**</td>
<td>R</td>
<td>13</td>
<td>44</td>
<td>4</td>
<td>-5</td>
<td>CON&gt;PSY</td>
</tr>
</tbody>
</table>

*p<.05; ** p<.01; ***p<.005
4.3.9 Seed-based functional connectivity analysis

Using FEAT from FSL, separate multiple regression analyses were performed in which we regressed each individual’s pre-whitened, scaled time-series against that of each of the ROIs. This resulted in participant-level maps of all voxels that were positively and negatively correlated with the seed ROI time-series. These were then converted to a Z-score using Fisher’s z transform.

4.3.10 Group-level analysis

A group-level functional connectivity analysis between the ChSz study participants (Contrasts: Con-A>Sz and Sz>Con-A) was conducted for the six ChSz study GO/NOGO task ROIs, five GO/NOGO combined group analysis ROIs, and one VBM conjunction analysis ROI. For the ABD study a group-level functional connectivity analysis between the ABD study participants (Contrasts: Con-C>AR and AR>Con-C) for the ten ABD study GO/NOGO task ROIs, five GO/NOGO combined group analysis ROIs and one VBM conjunction analysis ROI were conducted. There were a total of 12 seed ROIs for the ChSz study and 16 seed ROIs used in the ABD study analyses. The group-level analyses were accomplished by means of a mixed-effects ordinary least-squares model implemented in FSL. This produced thresholded Z-score maps of positive connectivity for each seed ROI. Correction for multiple comparisons was performed at the cluster level using the Gaussian random field theory (min Z>2.3; cluster significance: \( p<.05 \), corrected).

4.4 Results

4.4.1 Demographics

The ABD and ChSz study participants’ demographics have been previously described in Chapters Two and Three.
4.4.2 fMRI task behavioural performance and functional activity

The ABD and ChSz study participants’ performance and activity during the response inhibition task have been previously described in Chapters Two and Three.

4.4.3 Resting-state results

ChSz study

The schizophrenia patients and their matched controls revealed different patterns of connectivity. The Con-A group showed greater regional connectivity, in the frontal lobe, within the PFC, cingulate and striatal regions, and also greater fronto-parietal connectivity (Table 4.2). Figure 4.2 illustrates the regions that revealed greater connectivity in the Con-A group (Con-A>Sz), which included the left claustrum seed with the right middle frontal gyrus, ACC, and left lentiform nucleus (Figure 4.2A). An additional seed region in the right IFG exhibited connectivity with the right inferior parietal lobule (Figure 4.2B). In the Con-A group the right insula seed region, which revealed a reduction in GM volume in the PSY group, revealed greater connectivity with the left IFG (Figure 4.2C).

Figure 4.3 reveals the regions that had greater connectivity among the Sz group (Sz>Con-A). A seed region from the ChSz study STOPS analysis in the right posterior cingulate revealed greater RSFC with the right middle temporal gyrus (Figure 4.3A). Seed regions from the combined group STOPS analysis with greater RSFC in the Sz group were in the left middle temporal gyrus seed with the left thalamus and left ACC (Figure 4.3B), the left claustrum seed with the left postcentral gyrus (Figure 4.3C), and the left precuneus seed and left ACC/superior frontal gyrus (Figure 4.3D).
ABD study

The AR children did not produce the extensive connectivity networks as their comparison group, especially between frontal, parietal, cingulate, and striatal regions (Table 4.3). There were no seed ROIs from the combined group analysis which revealed group differences between the AR and Con-C groups. Figure 4.4 illustrates the seed regions from the ABD study STOPS analysis which revealed significantly more activity in the Con-C group compared to the AR children (Con-C>AR). These regions included the right IFG seed with the right rostral cingulate and another region in the right IFG, which extended into the middle frontal gyrus (Figure 4.4A, B). The left claustrum seed produced greater connectivity with the left dorsal ACC (Figure 4.4C). The right lentiform seed revealed greater connectivity with the right IFG (Figure 4.4D). The last region revealing greater connectivity in the Con-C group was within the parietal lobe in the right precuneus seed region with the right supramarginal gyrus (Figure 4.4E).

Figure 4.5 illustrates the seed regions from the ABD study STOPS analysis which revealed significantly more connectivity in the AR children compared to the control children (AR>Con-C). The AR group revealed increased RSFC in the right IFG seed to the left lingual gyrus (Figure 4.5A). The only other seed ROI revealing greater connectivity in the AR group was between the left claustrum seed and the right superior frontal gyrus (Figure 4.5B).

Figure 4.6 displays a schematic of the connectivity networks described in this ChSz and ABD studies separate “Results” sections. The Con-A group revealed greater connectivity in seed regions, which were also more active in the functional task, across hemispheres in mainly frontal lobe regions, particularly the cingulate, striatal. There was also right hemisphere fronto-parietal connectivity found. The Sz patients
Table 4.2
ChSz study participant’s resting-state functional connectivity results.
Seed regions showing significant differences in resting-state functional connectivity between adult controls (Con-A) and schizophrenia patients (Sz) (p<.05, corrected).

<table>
<thead>
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<th>x</th>
<th>y</th>
<th>z</th>
<th>Connectivity region</th>
<th>Side</th>
<th>BA</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Voxels</th>
<th>P</th>
<th>Z-score</th>
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<tr>
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<tr>
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</tr>
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</tr>
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### Table 4.3
ABD study participant’s resting-state functional connectivity results.
Seed regions showing significant differences in resting-state functional connectivity between control children (Con-C) and at-risk children (AR) \((p<.05, \text{corrected})\).

<table>
<thead>
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<th>Side</th>
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<th>Voxels</th>
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</table>
Figure 4.2. Connectivity maps reveal the regions of greater connectivity among the adult controls (Con-A) with the seed ROIs from the ChSz study and combined group analysis (Con-A>Sz) (p<.05, corrected). (A) Combined group STOPS Seed: L Claustrum → R Middle frontal gyrus and R ACC and L Lentiform nucleus. (B) ChSz Study STOPS Seed: R IFG → RN Inferior parietal lobule. (C) Conjunction VBM seed: R Insula → L IFG.

Figure 4.3. Connectivity maps reveal the regions of greater connectivity among the schizophrenia patients (Sz) with the seed ROIs from the STOPS analyses in the ChSz study and combined group analysis (Sz>Con-A) (p<.05, corrected). (A) ChSz Study STOPS Seed: R Posterior cingulate → R Middle temporal gyrus. (B) Combined group STOPS Seed: L Middle temporal gyrus → L ACC and L Thalamus. (C) Combined group STOPS Seed: L Claustrum → L Postcentral gyrus. (D) Combined group STOPS Seed: L Precuneus → L ACC/ Superior frontal gyrus.
Figure 4.4. Connectivity maps reveal the regions of greater connectivity among the control children (Con-C) with the seed ROIs from the STOPS analysis of the ABD study (Con-C>AR) (p<.05, corrected). (A) R IFG ➔ R IFG/Middle frontal gyrus (B) R IFG ➔ R Rostral cingulate (C) L Clastrum ➔ L ACC (D) R Lentiform nucleus ➔ R IFG (E) R Precuneus ➔ R Supramarginal gyrus.

Figure 4.5. Connectivity maps reveal the regions of greater connectivity among the at-risk children (AR) with the seed ROIs from the STOPS analysis of the ABD study (AR>Con-C) (p<.05, corrected). (A) R IFG ➔ L Lingual gyrus (B) L Clastrum ➔ R Superior frontal gyrus.
Figure 4.6. Overview of the intrinsic connectivity pathways which showed significant between-group differences in the ChSz and ABD studies. Red dots represent the seed ROI and a double-arrowed line connecting to the black dot correspond to the region showing connectivity to the seed ROI \( (p < .05, \text{corrected}) \). (+) Denotes a seed region with greater activity in that group during the STOPS task. (-) Denotes a seed region that was hypoactive in that group during the STOPS task. Note: The (-) represents a task deactivation in the left precuneus in the Sz group in the Sz>Con-C diagram. Also the (-) represents a deactivation in the Con-C group in the Con-C>AR diagram. (*) Represents the seed region derived from the VBM conjunction analysis which was reduced in the Sz patients.
however exhibited greater connectivity in seed regions, which were hypoactive during the functional task, in the left hemisphere in the temporal lobe, cingulate, thalamus and somatosensory regions. Echoing the adult controls, the Con-C group also revealed increased connectivity in regions that were hyperactive in the STOPS condition in frontal, cingulate, striatal and parietal regions. However, the AR children exhibited connectivity patterns within hypoactive task-related seed regions, in the IFG and claustrum, to occipital and superior frontal and regions.

4.5 Discussion

The aim of the present study was to use functionally- and structurally-defined seed regions to examine whole-brain resting-state connectivity. Schizophrenia has been characterised as a disorder of integration between brain regions (Bullmore, et al., 1997; Friston & Frith, 1995). Neuroimaging studies of functional activity have found the most consistent finding in chronic schizophrenia is hypofrontality during the task-active state (Hill, et al., 2004), especially during executive functioning tasks (Minzenberg, Laird, Thelen, Carter, & Glahn, 2009). The intra- and inter-regional relationships during the resting-state in the children and adult control subjects indicated greater cortical integration across the brain in the absence of a task. The schizophrenia patients revealed an almost exclusive pattern of left hemispheric connectivity, which was evidenced during the task of functional increases in the left and decreases in the right hemispheres. In the control children task-activated regions, such as the right IFG, claustrum and lentifrom nucleus, revealed greater RSFC with other well-known task-related regions, such as the right IFG, cingulate, and middle frontal gyrus. This indicates that an underlying increase in connectivity among these cognitive control regions, could contribute to the increased activation during the STOPS to overcome the demands of the task. These intra-regional correlations were
not observed in the AR group, who instead show increased RSFC within a region associated with the DMN, the superior frontal gyrus and a less mature brain region, the lingual gyrus in the occipital lobe.

4.5.1 Striatum connectivity

The lentiform nucleus, in the dorsal striatum, was the only region which showed greater connectivity in both the adult and control children but no connectivity in the Sz or AR groups. In the Con-A group the lentiform nucleus connected to the ipsilateral left hemisphere claustrum seed, and in the Con-C group the lentiform nucleus seed connected to the ipsilateral right hemisphere IFG. During the functional task the claustrum revealed increased activity in the Con-A group, and the lentiform nucleus had increased activity in the Con-C group. The Con-A group also had increased functional activity in the dorsal striatum region of the caudate; however, this region did not reveal increased connectivity. Therefore, functionally, both the AR and Sz groups revealed hypoactivity in the dorsal straitum. Hoptman and colleagues (2004) found that better GO/NOGO task performance was negatively correlated with a reduction in FA in the striatum. Casey and colleagues (1997) found disrupted fronto-striatal circuitry in children with ADHD during a response inhibition task. Their data suggests the role of the right PFC is to suppress responses to salient events, while the basal ganglia in the striatum appeared to be involved in executing the behavioural response. An underlying possible neurobiological explanation may be derived from the dopamine hypothesis of schizophrenia. This theory posits that the reduced dopamanergic tone found in the PFC is counteracted by the increased DA found in the striatum in schizophrenia patients. Abnormally high DA levels in the dorsal striatum have also been found in the unaffected siblings of schizophrenia patients (Huttunen, et al., 2008), individuals in the prodromal phase of the illness.
Chapter Four

Resting-state functional connectivity in children at-risk for psychosis

(McGuire, Howes, Stone, & Fusar-Poli, 2008) and ARMS individuals (Howes, et al., 2007). Therefore the lack of fronto-striatal connectivity coupled with the hypoactivity in the IFG and lentiform nucleus in the AR group could possibly be underscored by an increase in DA within the dorsal striatum. A theory proposed by Kapur (2003) has suggested that increased DA in the striatum causes salience misattribution in schizophrenia. They hypothesise that aberrant salience attribution leads to an inability to monitor and filter relevant from irrelevant stimuli. This serves as a distraction when it is necessary to carry out appropriate goal-directed behaviours. This could be a possible explanation for the AR and Sz patients reduced activity in the IFG, which leads to the inability to suppress the incorrect response, which leads to the inability of the dorsal striatum to carry out the appropriate action. In the adult controls the lentiform connects to the claustrum which connects to ACC, and middle frontal gyrus which are core components of the cognitive control processing.

4.5.2 Cingulate connectivity

A WM cingulum bundle region derived from DTI research in schizophrenia has revealed reduced connectivity between the cingulate gyrus and PFC in patients (Laurens, Ngan, Bates, Kiehl, & Liddle, 2003; Polli, et al., 2008). This bundle connects the PFC, cingulate gyrus, medial temporal lobe, including the hippocampus and parahippocampus, and precuneus (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005). WM of the cingulum has previously revealed FA reductions in schizophrenia patients within the PFC (Weiss & Heckers, 2001), cingulate gyrus (Fujiwara, et al., 2007; Kubicki, et al., 2003), and in the hippocampus in children and adolescents with schizophrenia (White, et al., 2007) and also in my investigation of children at-risk for psychosis (see Chapters Two and Three).
The left claustrum was a region that was hypoactive in both psychosis groups during the functional task. Interestingly, this region showed connectivity in all four groups during the resting-state. Although there was a notable consistency in the two control groups which both revealed greater connectivity from the claustrum seed region to the dorsal ACC, which is known as the cognitive subdivision of the ACC (Bush, et al., 1998). However, the psychosis groups show a different pattern of connectivity. The AR children reveal connectivity between the claustrum and contralateral superior frontal gyrus, which is similar to the pattern observed in the Con-A group of claustrum to contralateral middle frontal gyrus. However, the AR group did not reveal significant connectivity correlations to the ACC, which may be indicative of an early biomarker of disconnection in the at-risk brain which gives rise to a reduction in connectivity in the cognitive networks that relies on the dorsal ACC for its hypothesised role in conflict monitoring (van Veen, Cohen, Botvinick, Stenger, & Carter, 2001). However, during response inhibition the AR group displayed increased ACC functioning. This finding was also observed in an independent study of individuals at genetic and symptomatic risk for psychosis who revealed increased ACC activity during successful inhibitions (Allen, et al., 2009). The AR individuals activate the ACC to successfully complete the task; however, they did not reveal any connectivity in this area with the rest of the brain. Therefore, this may indicate that the cortical resources in the left ACC need to increase activity because this region is not supported with connections from other areas and hence the greater regional activity during the task. This is evidenced by the reduction in activity within the connected claustrum, which was hypoactive during the GO/NOGO task for the AR group. In regards to the Sz patients they also do not show connectivity from the claustrum to frontal or cingulate regions but instead claustrum connectivity with the
postcentral gyrus in the parietal lobe. This somatosensory region’s increased FC with the highly interconnected claustrum region could possibly explain the heightened sensory experiences of patients which are presented as positive psychotic symptoms.

4.5.3 Inferior frontal gyrus (IFG) connectivity in the ChSz study

The IFG located in the PFC is one of the most well-connected regions in the PFC (Miller & Cohen, 2001). A dysfunction in this region has the potential for a cascading effect on activity in the cortex. The Sz patients failed to exhibit connectivity with any regions in the frontal cortex whereas the Con-A group revealed connectivity within the right IFG and right inferior parietal lobe. This could be structurally explained by the finding of reduced FA in the right inferior parietal lobe WM in both the AR and Sz groups. The FA reduction fell along the SLF, a long association fibre connecting the frontal and parietal lobes. The SLF has been known to play an integral role in facilitating working memory functions (Petrides & Pandya, 2002). FA reductions along the SLF have been consistently found in schizophrenia patients (Buchsbaum, et al., 2006; Burns, et al., 2003; Hubl, et al., 2004; Jones, et al., 2006; Shergill, et al., 2007; Szeszko, et al., 2008) and at baseline examination in individuals at a high genetic risk (Karlsgodt, Niendam, Bearden, & Cannon, 2009). Reduced focal FA in the right parietal lobe along the SLF has been found in schizophrenia patients (Kaanan, et al., 2009) and in individuals at a high genetic risk for psychosis (Hoptman, et al., 2008). Although we did not find reduced right inferior parietal RSFC in the AR group, we did find a reduction in RSFC within the right parietal lobe, between the precuneus and supramarginal gyrus, in the AR children. Therefore, the parietal lobe reductions in RSFC in the AR children seem to fall in-line with those in Sz patients, but may be more diffuse during their developmental stage.
and become more specific and pronounced in the inferior parietal lobe over the course of adolescent development.

### 4.5.4 IFG connectivity in the ABD study

The children in the ABD study however presented a different pattern of RSFC with the right IFG. The IFG seed was hyperactive during the response inhibition task in the Con-C group and revealed greater connectivity with the known cognitive control networks of the ipsalateral cingulate and another nearby region in the ventral lateral IFG. This greater intra-regional connectivity can explain the increased and efficient functioning of the IFG in the Con-C group during the task. However, the AR children exhibited an alternative RSFC pathway from their task-related hypoactive right IFG with the left lingual gyrus, in the occipital lobe. Greater RSFC fronto-occipital connectivity has been observed in schizophrenia patients (Jafri, Pearlson, Stevens, & Calhoun, 2008). Additionally, the AR children revealed reduced FA in the right lingual gyrus, which might explain the increased connectivity with the contralateral lingual gyrus and the IFG. This also falls in-line with the greater activity observed in the left hemisphere in AR children, which revealed an over-reliance on the left hemisphere, giving rise to the inefficient inter-regional RSFC.

### 4.5.5 Precuneus connectivity

The right precuneus seed in the ABD study revealed greater connectivity in controls with another lateral parietal region of the DMN, the right supramarginal gyrus. The precuneus has been found to be core component of the DMN (Fox, et al., 2005; Fransson, 2005). The precuneus has also been found to be the single highest metaboliser of glucose in the entire brain (~35% of all glucose in the brain) (Gusnard & Raichle, 2001). Fox and colleagues (1988) found that during prolonged visual
stimulation cerebral blood flow to the visual cortex and glucose metabolism increased in nearly the same amount (50% and 51% respectively). The AR children revealed increased activity in the precuneus during the fMRI task, however both control groups and Sz patients all de-activated this region. Therefore, the AR children revealed an increase from baseline activity in this region. Since this region did not reveal increased resting-state connectivity this may be an indicator that they have reduced glucose in this region of the brain during rest, which leads to their inefficient recruitment of this area during the task instead of deactivating it, as the other groups were able to do.

4.5.6 Middle temporal gyrus connectivity

A region which revealed hypoactivity during the response inhibition task in the PSY group was the right middle temporal gyrus. However, the Sz patients revealed greater connectivity between the right middle temporal gyrus the ACC and also with the thalamus, which was not observed in any other group. One particularly important link with the subthalamic nuclei is from the IFG, which has shown to be important for motor inhibition (Aron & Poldrack, 2006). However, the Sz group did not reveal greater frontal lobe connectivity. Furthermore, the left thalamus has shown task-related functional connectivity with the basal ganglia, cerebellum, cingulate, and hippocampus, but not the temporal lobe (Stein, et al., 2000). The increased RSFC between the thalamus and middle temporal gyrus could explain the greater FA which was found in the putamen, an area that receives afferent inputs from the thalamus. Additionally, the left temporal lobe revealed GM increases in the Sz patients. The greater GM volume in the temporal lobe and increased WM surrounding the thalamus, may give rise to the increased connectivity between these two regions. However, this aberrant connectivity pattern of the thalamus, which does not follow normal cingulate
and hippocampal connectivity patterns, and instead temporal connectivity may reveal that these two regions are already active before task onset and do not increase activity with the increased demands required of the middle temporal region during the task. Additionally, the Sz group revealed greater connectivity between the middle temporal gyri and cingulate. Other studies have also found evidence in schizophrenia patients of greater task-related connectivity between temporal and cingulate regions using dynamic causal modelling connectivity (Allen, et al., 2009) and greater RSFC with the use of an ICA analysis (Jafri, Pearlson, Stevens, & Calhoun, 2008).

4.6 Conclusions

The RSFC analysis reveals great potential for assessing the risk for psychosis. Both the AR and Sz patients shared common patterns of aberrant RSFC organisation during the resting-state, which may underlie dysfunctional task-related activity. This analysis method has proven to be a sensitive tool in detecting Alzheimer’s and late-life pathological disease markers (Filippini, et al., 2009; Greicius, Srivastava, Reiss, & Menon, 2004); however, its scope in determining neurobiological markers in schizophrenia is still open to further experimentation. The current findings are the first to reveal RSFC networks in both schizophrenia patients and those at a heightened clinical risk for developing the disease.

The RSFC analysis compliments the DTI findings from the ChSz and ABD studies (see Chapters Two and Three) which suggested two networks of disturbed WM tracts in at-risk children and schizophrenia patients. These networks are the SLF which connects fronto-parietal regions and the cingulum bundle that connects frontal, cingulate, and parahippocampal regions. An abnormality in WM in this area reveals an important region where functional disintegration between frontal and posterior connectivity could be arise. These abnormalities may be subserved by WM
disruptions that were found in the PSY group along the connecting fibres of the fornix (in the parahippocampus) and anterior thalamic radiation (in the ventral striatum). The common regions of WM reduction in the parietal lobe and parahippocampus found in both psychosis groups could reveal the potential for a disconnection of the GM regions that they link which is observable before the onset of the first-episode and persists throughout the course of the illness. The fMRI findings of reduced functional activity in right hemispheric prefrontal, temporal, and clastrum regions in both those at-risk and schizophrenia patients reveals common functional task-related disturbances. With regard to structural connectivity, the FA results point to greater cortico-striatal-cerebellar connectivity within the PSY group, that likely arises due to abnormalities in frontal and temporal functioning. Additionally, the FA analysis revealed that the CON group had more efficient fronto-parietal and fronto-mesolimbic structural networks. Therefore, altered brain connectivity during the resting-state, which manifests at the macro-structural level during a functional task, which may be a result of micro-structural deficiencies along the WM tracts.

An obvious strength of the RSFC scans is the short scanning protocol times, roughly 3.5 minutes per resting-state scan. Additionally, the implementation of these scans will not rely on the cognitive abilities of the individual being tested. This serves to address another confound of imaging clinical populations, which is a sampling bias by only scanning patients who are cognitively able to complete the task. Whereas in resting-state individuals do not need to meet a priori behavioural performance criteria, nor do they even need to be awake or conscious to obtain reliable data (Fukunaga, et al., 2006; Vincent, et al., 2007).

The seed ROIs for the functional connectivity analysis were choosen to reduced inter-subject variability in the anatomical location of the seeds because they
were derived from the functional scans of the same subjects. The use of seed regions that revealed functional differences during the functional task has been shown to be an effective method of choosing seed regions for functional connectivity analyses (Xiong, Parsons, Gao, & Fox, 1999). However, this does not allow for an unbiased examination of functional regions of interest that are independent from the sample. Future, RSFC analyses will need to draw upon functional seed ROIs from meta-analyses using a task of interest. Additionally, seed regions in the DMN we not examined in this current study; therefore, in future work standardised seed regions within the DMN (Fransson, 2005; Smith, et al., 2009) should also be used to investigate established resting-state networks.

Due to the difficulty involved in the recruitment of the unique at-risk population and the limited access schizophrenia outpatients the sample size of these studies were relatively small and may have not allowed for the findings of more subtle differences in intrinsic connectivity patterns to survive the stringent statistical thresholds employed in my analyses.

Although resting-state connectivity has been shown to be a reliable method of correlating spontaneous neural activity (Gusnard & Raichle, 2001) the resting-state scans are affected by other signal sources. One such confound is normal physiological activities (i.e. slower breath-to-breath changes and heart rate) that affect the BOLD signal and increase noise during resting-state scans (Cordes, et al., 2001) revealing a strikingly similar pattern of deactivation as the default network (Birn, Murphy, & Bandettini, 2008). Birn and colleagues showed that low-frequency spontaneous neural activity (0 to 0.1 Hz) accounts for 90% of correlations across the brain, whereas similarly low frequencies in respiration (0.1 to 0.5 Hz) and cardiac fluctuations (0.6 to 1.2 Hz) had more correlations in blood vessels and cerebral spinal fluid. Therefore our
temporal resolution of two seconds would not be sensitive enough to filter out some physiological signals, which occur in \(~10\%\) of the resting-state correlations. However, we made attempts to increase the signal-to-noise ratio with the use of a number of nuisance regressors. In future studies, measurements of respiration and cardiac variations need to be measured and included in the model as additional nuisance regressors to account for physiological confounds.
5. Chapter Five
General discussion of the structural and functional neural correlates of the risk for psychosis
5.1 Summary of thesis findings: What does the vulnerability for psychosis look like in the brain?

Studying children experiencing psychotic symptoms provides a unique opportunity to examine vulnerability to psychosis within the context of development, which is free from confounds of medication and chronic illness. In Chapter Two multi-modal neuroimaging techniques were utilised to investigate cognitive control functions, brain morphometry and white matter integrity in an at-risk cohort of school children experiencing subclinical psychotic symptoms. Chapter Three drew upon the findings of Chapter Two and compared the neurobiological commonalities observed in those who are at-risk and those diagnosed with schizophrenia. In Chapter Four seed ROIs which exhibited group differences between psychosis groups and controls were used to investigate intrinsic functional connectivity pathways in the brain. The conclusion drawn from these investigations reveal that there are neurodevelopmental abnormalities found in the vulnerability stage which are also observed in the chronic state of the illness. Additionally, differences in cortical functioning and structure that were found only in the schizophrenia patients are believed to be disease-related and reflect the progressive neurodegeneration associated with the presence of the illness.

5.2 Structural and functional neural changes in children at-risk

The ABD study revealed the need to study the vulnerability to psychosis within the context of development to reveal regions of aberrant neural development before the onset of adolescence. Since adolescence and young adulthood is the most common window for the transition to a psychotic illness, the period preceding onset needs to be explored to elucidate focal brain abnormalities which may predict who will develop the illness.
Findings from the ABD study revealed that the developing brains of 11 to 12 year old children at-risk for developing psychosis show right lateralised fronto-temporal dysfunctions, as assessed during a task of response inhibition. The neuropsychological assessment session of the AR participant’s revealed diminished set-shifting, receptive language, and fine motor skill abilities. These tasks have been hypothesised to reflect fronto-temporal functioning. Reduced functioning in these regions was confirmed through the event-related analysis of the GO/NOGO task, suggesting these brain regions as potential neural correlates underlying the observed behavioural deficits.

Structurally, the left middle and superior temporal GM volume increases in the AR participants reveals that these regions may be sites of delayed neuronal pruning, as has been observed in individuals with childhood-onset schizophrenia (Jacobsen, et al., 1996). The hypothesis of delayed GM maturation is evidenced by studies showing a decline in volume during late childhood and throughout adolescence (Gogtay, et al., 2004). However, a reduction in the inferior temporal lobe found in the AR subject’s may be a specific vulnerability marker of psychosis, since this region has also shown reduction in a previous sample of individual’s with a high genetic-risk (Job, Whalley, Johnstone, & Lawrie, 2005).

WM micro-structural reductions found in the visual cortex, parahippocampus and left STG were observed in the AR group. The concurrent finding of increased GM and reduced WM integrity in the left STG in the AR group indicates a common pattern of aberrant temporal lobe neural development. Temporal lobe abnormalities have been consistently associated with positive symptoms in chronic schizophrenia (Kim, et al., 2003b; Shenton, et al., 1992; Turetsky, et al., 1995). A convergence between morphometry and reduced functioning, as observed during the GO/NOGO
task, suggests that the left temporal lobe may provide a particularly sensitive marker of early psychotic symptoms. The aberration within parahippocampal WM in the AR group could lead to memory difficulties, since this region is the first to receive sensory information before passing information through to the entorhinal cortex (Amaral & Price, 1984) and is thought to be a crucial substrate for memory acquisition, consolidation and retrieval (Fell, Klaver, Elger, & Fernández, 2002). The role of the parahippocampus in memory research has been observed in a PET study which found that the parahippocampus is recruited during the retrieval phase of visual spatial working memory tasks (Owen, Milner, Petrides, & Evans, 1996). Many of the prominent cognitive and behavioural deficits in schizophrenia can be conceptualised as a product of dysfunctional working memory, revealing that impairments in this ability have been conceptualised as a core feature of the illness (Cohen, Braver, & O'Reilly, 1996; Goldman-Rakic, 1994). Volumetric reductions within the parahippocampus have been observed in schizophrenia patients (Bogerts, Meertz, & Schönfeldt-Bausch, 1985) and individuals at a high genetic-risk (DeLisi, et al., 2006). A disruption in the WM fibres in the parahippocampus could lead to an inability to coordinate working memory functions, suggesting this brain region as a potential locus of this cognitive deficit.

These multi-modal findings provide a broad neurobiological characterization of individuals at symptomatic risk for developing psychosis. The results of the fMRI, VBM and DTI analyses support the hypothesis of frontal-temporal functional and structural deficits in these regions. Future corroboration could come from higher-resolution DTI data enabling delineation of the WM tracts, especially in long association fibres and also in the uncinate fasciculus (UF), which connects the IFG,
amygdala, hippocampus and temporal lobes, which in Chapter Three showed reduced FA in the Sz group.

5.3 Cross-sectional findings of structural and functional neural changes in children at-risk and schizophrenia patients

In Chapter Three I set out to systematically compare brain activity, morphometry and WM integrity between the AR children experiencing attenuated subclinical psychotic symptoms and adult, chronic schizophrenia patients with the full-blown illness. A combined group analysis of the GO/NOGO task identified overlapping regions of functional activity in the AR and Sz groups (PSY group). This analysis revealed a triad of hypoactivity within the prefrontal, temporal, and claustrum regions compared to the matched CON group. Additionally, one region that did not directly overlap in the combined group analysis but showed a reduction in the AR and Sz groups relative to control participants was in the dorsal striatum. The hypofrontality in the frontal lobe in the PSY group was localised in the right IFG and SFG regions. The other brain regions revealing functional deficits were the left middle temporal lobe and claustrum, which are brain areas known to be important in integrating semantic, linguistic, and sensory information (Calvert, 2001; Ettlinger & Wilson, 1990). Increased GM was found in the left temporal gyrus in the AR and Sz groups, and may be an underlying structural abnormality related to the reduced activity observed during the task. The PSY group had revealed hyperactivity in the precuens in the posterior brain region to perform the cognitive task. Additionally, separately the AR and Sz groups showed increased left DLPFC during the task relative to controls, even though this region did not directly overlap in the combined group analysis. This pattern of extraneous neural activity in the left DLPFC and parietal lobe supports the inefficiency hypothesis, which posits that hyperactivity is a
result of increased processing within the PFC to cope with the cognitive demands of the task and is evidenced by. The recruitment of posterior regions is also observed in the developmentally immature brains of children who show more diffuse, less active and widespread patterns of activation compared to adults during working memory and cognitive control tasks (Braet et al., 2009; Rubia et al., 2000; Thomas et al., 1999) and also in individuals at-risk for psychosis who experience psychotic symptoms (Whalley et al., 2004).

In my research into neurobiological risk markers for schizophrenia the DTI analyses revealed important clues into potential structural abnormalities sub-serving the reductions in task-related activity. In Chapter Two I presented DTI results for the AR children which revealed FA reductions within temporo-limbic-occipital WM tracts. The reduction along the cingulum bundle falling in the parahippocampus, in the limbic system, have been found in previous FA studies in schizophrenia patients (Agartz, Andersson, & Skare, 2001; Kalus et al., 2004; Kubicki, 2003; Wang et al., 2004). The cingulum bundle connects the PFC, cingulate gyrus, medial temporal lobe, including the hippocampus and parahippocampus, and precuneus (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005). FA reductions in WM in the PSY group were found along this fibre bundle in bilateral parahippocampus, in the WM connecting the two hemispheres of hippocampi, and posterior cingulate. Previous studies of WM fibre tracts in schizophrenia patients has revealed FA reductions along the cingulum in patients within the PFC (Weiss & Heckers, 2001), cingulate gyrus (Fujiwara et al., 2007; Kubicki et al., 2003), and in the hippocampus in children and adolescents with schizophrenia (White et al., 2007). The hippocampus has been consistently found to show volumetric reductions in schizophrenia patients (Harrison, 2004), however only deficits in WM integrity were found in my investigation. Goto and Grace (2008) have
studied rats to elucidate the neural mechanisms subserving the learning and memory processes involved in goal-directed behaviour. They found the hippocampus was involved in retrospective memory retrieval but additionally recruited the PFC to switch to a new memory strategy. This switching was facilitated by mesocortical DA. They hypothesised that hippocampal disruptions might produce an alteration in mesolimbic DA thereby indirectly interfering with mesocortical DA transmission in the PFC causing the observed inability to integrate goal-directed processes. Structural abnormalities in the hippocampus are believed to be a result of aberrant neurodevelopment, effecting synaptic organisation and neuronal connectivity, rather than neurodegenerative tissue damage, since it is observed in first-episode patients (Harrison, 2004). Although the Sz patients in this study did not reveal volumetric reductions in the hippocampus, they did have GM and functional reductions within the posterior cingulate, which falls along the cingulum, and could be an indicator of aberrant neural connectivity along the cingulum, which is related to the presence of the disease. No differences in posterior cingulate functioning, volume or WM integrity were found in the AR cohort.

The Sz patients revealed FA reductions in fronto-temporo-midline-cerebellar WM regions. The Sz patients revealed reduced FA in the IFG and middle temporal lobe along the superior longitudinal fasciculus (SLF), which was not found in the AR children. The SLF is a long association fibre that spans the WM tracts from the frontal to parietal lobes. The SLF has been show to play an integral role in facilitating working memory functions (Petrides & Pandya, 2002). The SLF is the most common fibre tract to show reduced FA in schizophrenia (Buchsbaum, et al., 2006; Burns, et al., 2003; Hubl, et al., 2004; Jones, et al., 2006; Shergill, et al., 2007) and at baseline examination in individuals at a high genetic risk (Karlsgodt, Niendam, Bearden, &
Cannon, 2009). The conjunction analysis of DTI data revealed that the SLF had reduced FA in the combined AR and Sz groups, which fell within the right inferior parietal lobe and right postcentral gyrus, which is also in the parietal lobe. The FA reduction in the right parietal lobe along this fibre tract was echoed in a recent study of a large sample (n= 76) of schizophrenia patients (Kanaan, et al., 2009). An abnormality in WM in this area reveals an important region where functional disintegration between frontal and posterior connectivity could arise, since frontal functional deficits were observed in the right hemisphere in the Sz patients and AR children.

Another well-replicated region of FA reduction found in the PSY group fell along the anterior thalamic radiation in the heavily DA mediated nucleus accumbens (NAcc) in the ventral striatum (Kanaan, et al., 2009). Our findings suggest two networks of disturbed WM tracts in at-risk children and schizophrenia patients: the SLF which connects fronto-parietal regions and the cingulum bundle that connects frontal, cingulate and parahippocampal regions. These abnormalities may be subserved by WM disruptions along the connecting fibres of the fornix and anterior thalamic radiation. A large meta-analysis of 407 schizophrenia patients concluded that FA reductions occur in two distinct regions: left frontal, along the cingulum and anterior thalamic radiation, and left temporal lobe, along the inferior fronto-occipital fasciculus and inferior longitudinal fasciculus (Ellison-Wright & Bullmore, 2009). These reductions were observed in both the ABD and ChSz studies. However, in the ChSz study the Sz patients revealed reductions in the left frontal cortex, which was not found to be impaired in the ABD study. Additionally, in the ABD study AR subjects revealed FA reductions in left temporal lobe, but this area was not found to differ in the ChSz study. Therefore, the reductions in the frontal lobe in the Sz
patients would seem to be related to the presence of the disease. However, the similar regions reduction in WM integrity in the parietal lobe and parahippocampus found in both psychosis groups could reveal the potential for a disconnection of the GM regions that they link which is observable before the onset and persists throughout the course of the illness.

The right anterior insula was the only region that showed a group difference between the PSY and CON groups, and was found to be reduced in the PSY group. Previous studies have reported the insula to have multiple and varied functions, such as playing a key role in the decision to respond or inhibit by analyzing affective information (Shafritz, Collins, & Blumberg, 2006), assessing emotional valence of affective stimuli (Kotz, Meyer, & Paulmann, 2006), and subjective awareness of bodily states (Craig, 2009). This paralimbic subcortical region is composed of agranular layers of neurons and has reciprocal connections with the amygdala and hippocampus via the ethorinal cortex (Amaral & Price, 1984). Carmichael and Price (1996) have posited that the insula integrates sensory and amygdaloid inputs. Neuroimaging research in macaque monkeys has revealed that the insula sends strong afferent projections to the ventral striatum, in the NAcc, which helps to integrate feeding behaviours with rewards and memory (Fudge, Breitbart, Danish, & Pannoni, 2005). This coupling of visceral and cognitive processes has also been observed in addiction research.

Reductions in insular volumes have been previously reported in schizophrenia patients at different stages of the illness (Honea, Crow, Passingham, & Mackay, 2005; Shapleske, Rossell, Simmons, David, & Woodruff, 2001; Kasai, et al., 2003; Sigmundsson, et al., 2001; Wright, et al., 1999), in those with a genetic-risk (Meisenzahl, et al., 2008) and those at-risk who convert (Borgwardt, et al., 2007;
Takahashi, et al., 2009). Their study suggested that reductions in insular cortex GM may reflect pre-existing vulnerability for psychosis, and is also a region of neurodegenerative progressive during the transition period into psychosis. In Chapter Two I reported isruptions in insula functioning in the AR group during unsuccessful inhibitions during the GO/NOGO task. Since unsuccessful trials were not examined in the Sz patients, due to a lack of a sufficient number of error trials, insular functioning was not measured. The recruitment of the insula has been shown in other tasks of attention and inhibition (Kirino, Belger, Goldman-Rakic, & McCarthy, 2000), indicating that the insula may become activated when the increasing demands of the inhibition task become greater than one’s ability to sustain their attention.

My results confirm previous theories of schizophrenia that highlight the existence of abnormalities in the brain, which manifest at the macro-structural and functional level, and could be a result in disconnectivity in the brain due micro-structural changes in WM tracts (Friston & Frith, 1995). The reductions in activations in right frontal and temporal regions during the inhibition task in the PSY group, may be a result of the abnormal micro-structural WM sub-serving these regions found along the cingulum bundle which connects the PFC to the limbic system. Also, a deficit in the ventral striatum, which was found in the NAcc in the PSY group, could be an indicator of aberrant DA transmission in the ventral striatum, which has been suggested in the dopamine hypothesis of schizophrenia. Additionally, a disruption in the WM in the fornix, connecting the two hippocampi, and in the bilateral parahippocampi could cause further disruptions to the mesolimbic pathway, which may in turn, give rise to memory deficits. Lastly, with respect to structural WM connectivity across the brain FA results point to greater fronto-parietal and fronto-limbic WM integrity in the CON group and greater dorsal striatal-cerebellar WM...
integrity within the PSY group may be hypothesised to be a compensatory WM increase. This pattern of FA decreases in the PSY group may lead to the frontal and temporal dysfunctions observed during the functional task.

5.4 Resting-state functional connectivity in children at-risk

In Chapter Four I reported the RSFC analyses which show great potential in assessing intrinsic connectivity in the brain in individuals at clinical risk for psychosis. The regions revealing decreased connectivity in the AR and Sz groups also revealed hypoactivity during the response inhibition task. We found in both the AR and Sz patients that there were common patterns of aberrant functional connectivity during the resting-state which may underlie the dysfunctional task-related activity. These findings are the first to reveal RSFC networks in both schizophrenia patients and those at a heightened symptomatic-risk for developing psychosis.

The aim of the RSFC investigation was to use functionally and structurally defined seed regions to examine whole-brain resting-state connectivity. Schizophrenia has been characterised as a disorder of integration between brain regions (Bullmore, Frangou, & Murray, 1997; Friston & Frith, 1995). The intra- and inter-relationships between brain regions in control subjects indicated greater cortical integration. The schizophrenia patients revealed an almost exclusive pattern of intra-regional connectivity in the left hemisphere only, which was evidenced during task-related activation by activity increases in the left and decreases in the right hemispheres. Healthy control children revealed that regions activated during the task show greater connectivity with other task-positive regions, such as the IFG, cingulate, and middle frontal gyrus, during the resting-state. Indicating increased connectivity among these cognitive control regions could aid in greater activation of these regions to successfully perform the task. The strength of these intra-regional correlations was
reduced in the AR group. The AR children did not reveal intra-regional connectivity and instead, showed increased connectivity within a region associated with the DMN (superior frontal gyrus) and a less neurodevelopmentally mature brain region, which was the lingual gyrus in the occipital lobe (Giedd, et al., 1999).

The lentiform nucleus seed region, in the dorsal striatum, showed reduced connectivity in both the AR and Sz groups. During the functional task both the AR and Sz groups revealed hypoactivity in the dorsal striatum. The AR children revealed reduced connectivity between the lentiform nucleus seed and the right IFG. Hypoactivity during the response inhibition task was found in the IFG and lentiform nucleus in the AR group, this hypoactivity could possibly be underpinned by decreased fronto-striatal connectivity. The Sz patients revealed reduced lentiform nucleus connectivity with the claustrum. The claustrum seed showed reduced connectivity with the ACC and also the right middle frontal gyrus, which are core components of the cognitive control processing. The Sz patients revealed hypoactivity in right PFC, cingulate, claustrum, and striatum, which may be a cause of the reduced connectivity between these regions, which leads to the poorer performance observed in the Sz patients during the response inhibition task.

The left claustrum was hypoactive in both of the psychosis groups during the functional task. Interestingly, this region showed connectivity in all four groups during the resting-state. However there was a notable consistency in the two control groups which both revealed greater connectivity from the claustrum seed region to the dorsal ACC, which is known as the cognitive subdivision of the ACC (Bush, et al., 1998). In regards to the Sz patients also did not show increased connectivity with the claustrum to the postcentral gyrus, which corresponds to the somatosensory brain region located in the parietal lobe. This somatosensory region’s increased
connectivity with the highly inter-connected claustrum region could possibly explain the heightened sensory experiences of patients which are presented as positive psychotic symptoms. The AR children revealed increased connectivity between the claustrum and contralateral superior frontal gyrus, which was similar to the pattern observed in the Con-A group of claustrum to contralateral middle frontal gyrus.

The AR group did not reveal significant connectivity correlations to the ACC, which may be indicative of an early biomarker of disconnection in the at-risk brain which gives rise to a reduction in connectivity in the cognitive networks that relies on the dorsal ACC for its hypothesised role in conflict monitoring (van Veen, Cohen, Botvinick, Stenger, & Carter, 2001). However, during response inhibition the AR group displayed increased left ACC functioning, which may indicate that functioning in this area is not supported by connections from other brain regions, hence the greater activity level in the left ACC in the AR group.

Dysfunction in the IFG located in the PFC, and is one of the most well-connected regions in the brain (Miller & Cohen, 2001), has the potential for a cascading negative effect on activity in the cortex. The Sz patients failed to exhibit connectivity with any regions in the frontal cortex whereas the Con-A group revealed connectivity within the right IFG and right inferior parietal lobe. This could be structurally explained by the finding of reduced FA in the right inferior parietal lobe WM in both the AR and Sz groups. Although I did not find reduced right inferior parietal RSFC in the AR group, there was a reduction in the AR group in intra-regional connectivity within the right parietal lobe (between the precuneus seed and the supramarginal gyrus). Therefore, the parietal lobe reductions in RSFC in the AR children seem to fall in-line with those in Sz patients, but may become more pronounced in the inferior parietal lobe over the course of adolescent development.
The control children in the ABD study presented a distinct pattern of RSFC with the right IFG, which was different from the adult controls. The IFG seed revealed increased activity during the response inhibition task in the Con-C group and revealed greater connectivity with the known cognitive control networks of the ipsalateral cingulate and another nearby region in the ventral lateral IFG. This greater intra-regional frontal connectivity in the Con-C group could explain the greater functional activity of the IFG group during the task. However, the AR children exhibited an alternative connectivity pattern from the controls. The AR group had greater right IFG connectivity with the left lingual gyrus, in the occipital lobe. Greater fronto-occipital RSFC has been observed in schizophrenia patients (Jafri, Pearlson, Stevens, & Calhoun, 2008). The increased activity in the left hemisphere in AR children during the functional task could be due to an over-reliance on left hemisphere brain regions due to the inefficient inter-regional connectivity.

The left middle temporal gyrus seed in the Sz group was a brain region that was hypoactive during the response inhibition task, but showed greater connectivity with the thalamus and ACC. The left temporal lobe revealed grey matter increases in the Sz and AR groups. Increased left hemisphere activity in the Sz patients could be due to the increased GM volume in the left temporal lobe and increased left hemispheric cingulate and thalamic connectivity.

The resting-state results reveal connectivity patterns that may underscore the hypoactivity in the psychosis groups within the right PFC, claustrum and dorsal striatum. Sz patients do not reveal increased connectivity in the frontal lobe, however, the AR children reveal increased right PFC connectivity, therefore frontal connectivity reductions could be related to neurodegenerative processes associated with the disease and are not identifiable in the risk stage. The AR group did not reveal
increased connectivity within the ACC, however the Sz group did, which may reveal a brain region which is related to the vulnerability for developing schizophrenia, but not observable in those with the established illness. Reduced dorsal striatum connectivity appeared in both the AR and Sz groups and could possibly serve as an early risk biomarker for the disease.

5.5 Limitations and future directions

The ABD study used a screening tool which was shown to have strong predictive validity in those who later convert to schizophrenia (Poulton, et al., 2000). However, this initial assessment included questions probing positive symptomatology, but not the negative or disorganised symptoms that are associated with the disease. A more comprehensive screening approach, which assesses for positive and also negative symptoms, would allow for more stringent inclusion criteria of at-risk individuals who exhibit a wider spectrum of symptoms that more closely resemble patients diagnosed with the disease. For example, population-based study in London (Laurens, West, Murray, & Hodgins, 2008) compared 6,008 children aged 9-12 years old who were experiencing psychotic-like symptoms to children free of psychotic-like symptoms. They used empirically based inclusion criteria for their at-risk group in which children had to meet all three of the following criteria: caregiver reports of abnormal speech or motor development in the child; caregiver reports of social, emotional, or behavioural problems in the child; and child reports of psychotic-like experiences. Children meeting the at-risk criteria revealed poorer performance on cognitive tests, including verbal working memory and response inhibition tasks. These inclusion criteria should be considered in future population-based research in identifying individuals at-risk, since they assess both the positive and negative symptomatology associated with schizophrenia.
Future neuroimaging studies in at-risk groups will need to explore fully the presence of subclinical psychotic symptoms, in particular the most frequent symptom of auditory verbal hallucinations. The GO/NOGO task was not intended to recruit temporal language regions, nor elicit hallucinations while in the scanner. Future research will need to use tasks mimicking the "voices" they hear, to see if our finding of increased GM in the temporal and angular regions is related to increased activity in the aforementioned regions. Future at-risk investigations should also include tasks which require the processing of emotional valence, to target insular and amygdala functioning, and address if the volumetric reduction in the insula could underlie blunted emotional states which have been observed in chronic patients. The Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) initiative (Barch, Braver, Carter, Poldrack, & Robbins, 2009) is currently working to identify a reliable battery of cognitive tasks which elicit specific cognitive dysfunctions in schizophrenia patients across distinct cognitive systems, which includes the response inhibition stop signal task. Once a stable battery of cognitive tasks and normative values are established I believe that will introduce a new wave of empirical research which provides consistent findings across the development stages to the progression to psychosis, which can be complemented by investigations with animal models, neuroimaging modalities, genome wide association studies and varied levels of risk populations.

Future DTI studies with risk groups should have high enough spatial resolution, which would need to be in 60 directions or more, to facilitate a tractography analysis which would allow for a more accurate assessment of neuronal functional connectivity. In particular, one could isolate an abnormality in FA and follow the specific tract it falls on to see if any the difference in GM, functional and
structurally, fall between the connecting WM tracts. Additionally, with a higher number of diffusion gradients one could more thoroughly differentiate the complexity of the organisation of WM tracts, to observe where changes in FA are falling on a particular tract and to disentangle the inherent issue of crossing fibres.

In Chapters Three and Four I reported on chronic schizophrenia patients, all of which were on stable neuroleptic treatment, whereas none of the at-risk children were medicated. Due to the nature of the subclinical psychotic symptoms in the children none of them were candidates for pharmacological intervention, nor therapeutic treatment. Therefore, although the psychosis groups were not an ideal “patient control” group of due to medication confounds, this group did serve to establish brain regions that were aberrant in a sample of patients with the established disease using parallel analyses. Future longitudinal studies of risk groups need to include an unmedicated first-episode patient control group along with medicated patients to disentangle the effects of medication on the brain structure and function, in addition to the inclusion of an un-medicated at-risk group.

Although resting-state connectivity has been shown to be a reliable method of correlating spontaneous neural activity (Gusnard & Raichle, 2001) the resting-state scans are affected by other signal sources. One such confound is normal physiological activities (i.e. slower breath-to-breath changes and heart rate) that affect the BOLD signal and increase noise during resting-state scans (Cordes, et al., 2001) revealing a strikingly similar pattern of deactivation as the default network (Birn, Murphy, & Bandettini, 2008). Birn and colleagues showed that low-frequency spontaneous neural activity (0 to 0.1 Hz) accounts for 90% of correlations across the brain, whereas similarly low frequencies in respiration (0.1 to 0.5 Hz) and cardiac fluctuations (0.6 to 1.2 Hz) had higher correlations in blood vessels and cerebral spinal fluid. Therefore
our temporal resolution of two seconds would not be sensitive enough to filter out some physiological signals, which occur in ~10% of the resting-state correlations. However, we made attempts to increase the signal-to-noise ratio through the inclusion of a number of nuisance regressors which aimed to capture uninteresting sources of signal variation. In future studies, measurements of respiration and cardiac variations need to be conducted and included in the model as additional nuisance regressors to account for physiological confounds.

An unavoidable limitation of the studies presented in this thesis are the small samples sizes of the studies, due to factors such as recruitment resources, willingness of targeted populations to participant and their caregivers consent, and time and monetary constraints on data collection. Although the ChSz study had a sufficient amount of participants for the analysis of the response inhibition task, based on previous power calculations for this task (Murphy & Garavan, 2005), the ABD study did not have as much statistical power as would have been ideal. Power calculations (Cohen’s $d$) were used to investigate some of the more subtle effects which I anticipated to see in the ABD study, for example group differences within the inferior parietal lobe during STOPs, required a sample size of N=152 participants to reach significance between groups. Therefore results found between the at-risk and control children reveal highly significant results, even with the small sample size. Future data in clinical high risk samples also need to have sufficient numbers of participants to allow for the fact that the majority of individuals (~35-84%) experiencing psychotic symptoms do not develop psychosis (Hanssen, Bak, Bijl, Vollebergh, & van Os, 2005; Poulton, et al., 2000; van Os, Linscott, Myin-Germeys, Delespaul, & Krabbendam, 2009).
The at-risk group identified in the ABD study should not be conceptualised as a “prodromal” cohort, but rather a group of non-treatment seeking children who exhibit a risk phenotype based on the presence of subclinical psychotic symptoms. While there is an increased risk for clinical psychosis among children reporting subclinical psychotic symptoms, the majority of individuals experiencing psychotic symptoms do not develop psychosis. A meta-analysis conducted by van Os et al. (2009) found indicates that approximately 75-90% of developmental psychotic experiences are transitory and disappear over time. Future work should concentrate on longitudinal assessments of pre-pubescent children who report subclinical psychotic symptoms and assess functional and structural measures at regular intervals between baseline and transition to the illness, therefore removing the Type II errors, which may contaminate the results of the current study. Additionally, one could isolate regions in the brains of those who do not convert that might protect them against the conversion to the illness. These could serve as key regions to target in therapeutic interventions during the risk state. Findings within this at-risk group add to our greater understanding of how brain variation at an early age, and perhaps at an earlier age then previously assumed to be detectable, can relate to the development of psychotic symptoms.

The time-frame of this research did not lend itself to the longitudinal investigation into what individuals in the AR group will develop psychosis in later years. This investigation would validate whether the abnormalities observed are related to the vulnerability to develop the illness or are indeed early markers of the development to the disease. A longitudinal assessment of children at-risk is required to glean this information. To address, I have begun work on identifying a new cohort of children who are experiencing psychotic-like symptoms, who are currently
undergoing scanning and will be conducting a longitudinal follow-up in three years. A preliminary report of this study, entitled the IMAGEN-Psy study, is presented in Appendix Ten.

A region that appeared in the DTI analysis, but not in the functional results was the NAcc in the ventral striatum. Assessment of functioning in this region is important to understanding motivation and salience attribution in individuals who are at-risk, due to impairments in these factors in schizophrenia patients. The IMAGEN-Psy MID task will potentially be able to elucidate the activation differences in this region between at-risk subjects and controls.

Although the research in this dissertation has identified focal regions of abnormalities in the children at-risk, the underlying neurobiological mechanism driving these functional, structural and connectivity anomalies is unknown. I have alluded to the role of DA throughout the thesis to emphasis the dominant dopamine hypothesis of schizophrenia, yet I did not have a direct measure of DA uptake and transmission. Future research in at-risk cohorts of children experiencing psychotic-like symptoms will need to follow in the footsteps of Howes and colleagues (2009) who have taken a mutli-modal approach of studying risk through the simultaneous use of PET and fMRI.

5.6 Final comments

Bechdolf and colleagues (2006) described the emerging global focus on identifying psychosis in the earliest stages, with the main focus of preventative intervention in schizophrenia, which has increasingly become a focus of psychiatric research interests. This work highlighted the need to improve the identification of pre-psychotic symptoms. They argued that CBT may have some advantages compared with antipsychotics in the early treatment and possible prevention of developing
schizophrenia. However, they concluded that ethical issues exist in judging whether psychological intervention is a realistic option for the treatment of people at risk of psychosis. This thesis did not set-out to identify pre-psychotic symptoms, as in such previous influential studies (McGlashan, Miller & Woods, 2001; Yung, et al., 2003), but rather intended to identify possible neurobiological abnormalities related to the presence of attenuated psychotic symptoms which seem to amplify nearer to the onset of the illness and persist throughout the illness until controlled through neuroleptic and therapeutic means. These neurobiological risk-related regions of the brain, which are intimately intertwined to allow cognitive functioning, can serve as key targets in cognitive remediation therapy in children who are identified as at-risk. Brekke et al. (2009) carried out a successful study of a potential intervention strategy which used empirically derived data from the cognitive research. This was a community-based psychosocial intervention study in schizophrenia patients which examined the magnitude of neurocognitive outcome over the space of only one year period of treatment. They found that 58% of the patients showed improved neurocognition over time. Patients with neurocognitive improvement also showed functional improvement that was 350% greater than patients who neurocognitive skills did not improve. This study reveals optimism in the ability to implement targeted neurocognitive interventions to help improve functional and treatment responsiveness for individuals with schizophrenia.

Whether that risk is genetic or the experience of attenuated psychotic symptoms, or a synthesis of both needs further examination from longitudinal in vivo investigations of the intricate connective web in the brain. We are only beginning to touch on the complexity of this illness through this research, which needs to continue to determine the necessary time to intervene and the most direct method to use to
combat this disease of the brain which brings devastation to the lives it touches and leaves researchers mystified in its wake.
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7. Appendices
Appendix One

An fMRI investigation of a novel analogue to the Trail-Making Test to examine cognitive flexibility in schizophrenia

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Abstract

The Trail Making Test (TMT) is a widely used neuropsychological measure that assesses visuomotor abilities and cognitive flexibility. For the TMT-A condition participants are required to locate and connect numbers (i.e. 1-2-3...) while in the TMT-B condition participants perform the set-shifting task of locating and connecting numbers and letters (i.e. 1-A-2-B...). The TMT-B condition has shown impairments in many clinical populations, including frontal lobe lesion and schizophrenia patients, but the neurobiological underpinning of the task can be difficult to discern given pragmatic obstacles in adapting the task for neuroimaging. Experiment one demonstrated a close correspondence between performance on the TMT and a novel, computer programmed adaptation of the TMT (pcTMT). The pcTMT was designed for functional imaging and functional Magnetic Resonance Imaging (fMRI) data were obtained in Experiment two. A whole brain comparison revealed significantly greater activation during the pcTMT-B relative to the pcTMT-A in right inferior/middle frontal cortices, right precentral gyrus, left angular gyrus/left middle temporal gyrus. These results identify the regions that exhibit cognitive flexibility impairments during the TMT. Future neuroimaging research utilising the easily administered pcTMT needs to be carried out in clinical populations that are known to have impaired set-shifting abilities.
Introduction

The Trail Making Test (TMT) has become a hallmark of numerous clinical and research-based testing batteries since its inclusion in the esteemed Halstead-Reitan Neuropsychological Test Battery (Reitan, 1955). The TMT has been used in well over 1,000 published studies and is possibly the most economical measure for neuropsychological screening (Horton, 1979; Reitan, 1955). The test consists of two parts: Part A (TMT-A) and Part B (TMT-B). In TMT-A participants are shown a random scattering of the numbers 1 through 24 on a page and are asked to connect the numbers in ascending order by drawing a line between them (1-2-3...). The TMT-B is the more difficult condition in which the visual presentation contains letters and numbers and participants serially alternate between the two types of stimuli connecting numbers and letters in order (1-A-2-B...). The TMT-A is used as a baseline measure of visual search and motor functioning. The total time to complete each condition is recorded and a difference score, TMT-B minus the baseline TMT-A, creates a standard and quantifiable measure of the executive processes involved in cognitive set-shifting, thus isolating the executive components engaged during the TMT-B.

Set-shifting between stimuli occurs when endogenous attention is guided to distinctive or locally-contrasting visual features, which are relevant to completing a given task (Wolfe & Horowitz, 2004). In our daily lives we must exercise the ability to visually adapt to a range of stimuli co-occurring in our environment. Whether it is searching for our new car in the parking lot or separate socks in the laundry, we are required to consciously control our visual system to allow processing resources to be engaged in goal-directed behaviours. Rogers and Monsell (1995) have posited that success in shifting attention lies in one’s ability to flexibly adapt and focus attention
to dynamic changes. The TMT exemplifies the attention shifting phenomenon and is a standardised way to measure visuoconceptual and visuomotor tracking skills, concentration on psychomotor speed, divided attention, mental flexibility, and the ability to shift cognitive sets all in one simple test (Reitan, 1958). Impaired set-shifting during the TMT-B has been observed in many clinical illnesses including, eating disorders (Holliday, Tchanturia, Landau, Collier, & Treasure, 2005; Roberts, Tchanturia, Stahl, Southgate, & Treasure, 2007), attention-hyperactivity disorder (Boonstra, Kooij, Oosterlaan, Sergeant, & Buitelaar, 2005), substance abuse disorders (Horton & Roberts, 2002), obsessive compulsive disorder (Moritz, et al., 2002), frontal lobe lesion patients (Stuss, Binns, Murphy, & Alexander, 2002), and schizophrenia patients (Gold, Carpenter, Randolph, Goldberg, & Weinberger, 1997).

The ability to exert cognitive flexibility, or cognitive control, is one of the most advanced abilities of the human brain (Braver, Reynolds, & Donaldson, 2003). The ability to flexibly and simultaneously adapt and allocate cognitive resources during a given situation allows humans to carry out intended goal-directed behaviours. The cortical locus of cognitive control has consistently been implicated in the prefrontal cortex (PFC) through lesion and in vivo neuroimaging studies. The PFC has been shown to reveal extensive communication with nearly every cortical and subcortical region (Miller & Cohen, 2001). This extensive reciprocity identifies the PFC as playing a critical role in monitoring and processing cortical activities (Fuster, 1997). Neuroimaging research has identified a specific region in the brain, the dorsal lateral prefrontal cortex (DLPFC), to be the central orchestrator of top-down executive processing in the brain (Fassbender, et al., 2009; MacDonald, Cohen, Stenger, & Carter, 2000; Smith, Jonides, Marshuetz, & Koepepe, 1998).
The “guided action” theory of cognitive control has become a prominent theory of executive functioning, which states that cognitive control is a phenomenon which emerges from the ability to actively represent current behavioural goals, or “context” (Cohen & Servan-Schreiber, 1992). Context processing relies heavily on working memory abilities to hold these representations on-line and flexibly adapt and bias behaviour to achieve current task goals. The PFC in this theoretical framework is believed to be engaged in the construction, maintenance, and manipulation of internal representations of context, through guiding processes within task-specific pathways in the brain, favouring processing occurring along task-relevant paths as opposed to task-irrelevant paths. Continuous performance tests (CPT), for example the AX-CPT, require participants to detect a target event among a serial string of briefly presented stimuli, while avoiding responding to distracter stimuli. Schizophrenia patients, and often their unaffected relatives, show lower target accuracy, or hit rates, and higher incorrect responses to distracters, or false alarms, compared to controls during this task (Cohen, Targ, Servan-Schreiber, & Spiegel, 1992). A PET study revealed that abnormal metabolism in the PFC of schizophrenia patients occurs during context processing tasks (Weinberger, Berman, & Zec, 1986). This seminal study revealed that behavioural deficits observed in schizophrenia patients may be subserved by a neurochemical dysregulation in the PFC. These neurobiological and functional findings are consistent with the dopamine hypothesis of schizophrenia which posits that the PFC, which is a primary projection area for the mesocortical dopamine (DA) system, experiences increased mesolimbic DA activity and may result in hypofrontality and the cognitive deficits observed that lie at the core of the disease (Weinberger & Berman, 1988).
Cognitive functioning and the PFC

Studies of brain development across the lifespan have revealed that the PFC is the last cortical region to complete development. Changes in cognition are believed to coincide with developmental changes in the brain, specifically shifting from diffuse to focal cortical engagement during cognitive challenges with increases in age (Durston, et al., 2006). Recent neuroimaging studies in pre-adolescent children have revealed that they exhibit more diffuse activation of the PFC during cognitive tasks, suggesting that less-efficient or less well-developed networks are being engaged during specific processing (Luna, Garver, Urban, Lazar, & Sweeney, 2004). In this thesis investigation of PFC functioning in children at-risk is studied with a cognitive control test of response inhibition (Caravan, Ross, & Stein, 1999). Revisiting the guided action theory, inhibitory control can be theorised to be engaged during cognitive control to override competing and interfering representations and processing pathways, allowing for the suppression of inappropriate behaviours.

TMT performance in schizophrenia

Neuroimaging studies within clinical populations have mainly used performance during the paper-and-pencil TMT as a proxy measure of impairment in cognitive control, which can be mapped on to localized brain abnormalities. Researchers have attributed longer TMT-B response times (RT) to focal activations in the brain. A study of schizophrenia patients investigated resting-state brain metabolism and found hypometabolism in bilateral frontal (superior, middle, and inferior) lobes and hypermetabolism in temporo-parieto-limbic regions predicted poorer performance on the TMT-B (Horacek, et al., 2006). Suggesting that dysfunctional connectivity between the fronto-temporal lobes may underlie aberrant TMT-B performance. Another study examining chronic schizophrenia patients linked
poor performance on the TMT-B with structural magnetic resonance imaging (MRI) findings of reduced prefrontal cortical grey matter (GM) (Bonilha, et al., 2008). This finding validated previous research that showed a reduction in PFC GM correlated with impaired set-shifting performance in schizophrenia patients (Lawrie & Abukmeil, 1998). This research complements the hypothesis that losses in frontal cortical GM have an adverse effect on cognitive performance, which are particularly pronounced when engaging in demanding executive set-shifting tasks. A behavioural study of attentional set-shifting and spatial working memory in first-episode and chronic schizophrenia patients revealed that chronic patients reveal attentional set-shifting and spatial working memory deficits but first-episode patients were only impaired on the working memory task, but not the set-shifting task, compared to healthy controls (Panetlis, et al., 2009). Their study has suggested that the impairments in attentional set-shifting are intact at onset and deteriorate due to neurodegenerative processes, whereas the working memory deficits are a result of incomplete neuronal maturation alluding to a neurodevelopmental cause prior to illness onset. A study of individuals in the at-risk mental state (ARMS) who later converted to psychosis revealed that ARMS participants had intact sustained attention functioning (Francey, et al., 2005). Other studies of individuals in the ARMS who later converted to psychosis exhibited performance deficits at baseline which subsequently worsened at a 12-month follow-up on the cognitive tests of TMT-B, visual reproduction memory (Wood, 2007), and verbal memory (Lencz, et al., 2006). These data suggest that the impairments in attention are intact at onset and deteriorate after prolonged duration of the illness due to neurodegenerative processes. However the deficits in working memory and set-shifting abilities on the TMT-B are observable at onset and could be a result of incomplete neuronal maturation, which alludes to an aberrant neurodevelopmental
course. Neuroimaging assessments of set-shifting abilities in pre-psychotic individuals are needed to elucidate if this deficit is identifiable during the risk stage preceding onset and if so, where does this deficit originate in the brain.

**Neuroimaging the TMT**

The first study to use the TMT during functional magnetic resonance imaging (fMRI) utilised a verbal adaptation of the TMT, which was comprised of a covert articulation of the Trails A and B, to make the task suitable for neuroimaging (Moll, de Oliveira-Souza, Moll, Bramati, & Andreiuolo, 2002). Results from this study revealed left localized dorsal lateral and medial prefrontal areas of activation during Trails B. Although the study is pioneering in its efforts to functionally image the TMT, the absence of the visual component of the task and the novel scoring method of utilizing a self-report from the participants of their highest number and letter reached in their best trial, instead of the RT, to derive the total score, limits the task’s ability to retain the same neuropsychological properties of the original TMT (Ruchinskas, 2003). Zakzanis and colleagues (2005) set out to optimise the TMT for fMRI by removing the verbal component of the aforementioned study. To stay true to the paper-and-pencil TMT they created a device called the “virtual stylus,” an MR-compatible manipulandum that captures the motor component of the TMT. Zakzanis’ main findings were similar to Moll’s et al. (2002) with the addition of activations within right lateralised cingulate and paracentral lobule when Trails A was subtracted from Trails B. However, on a practical level, Zakzanis’ TMT writing apparatus might pose some obstacles for most imaging centres, such as the cost and availability of the device, and also has the potential to introduce motion artefacts into the fMRI environment. Even under optimal conditions and high magnetic field strengths, head movements may easily corrupt the activation results, and in the worst case involve
losing a subject’s data due to excessive motion (Friston, Williams, Howard, Frackowiak, & Turner, 1996).

The following chapter describes an easy-to-administer, novel computer-based analogue of the TMT (the pcTMT) that retains the set-switching properties of the original test. Experiment one examined the correspondence in behavioural performance between the TMT and the pcTMT. The second experiment tested the pcTMT in a healthy sample of participants during an fMRI scan.

Experiment one

Materials and methods

Participants

Twenty healthy participants (10 males; Mean age: 22.7±3.7 years, range 18-33 years; 18 right-handed; education 17.4±1.9 years, range 14-20 years) participated in the behavioural study. All of the participants spoke English as their native language. The participants were given a brief measure of intelligence that was assessed by the WRAT-4 reading subscale (Mean score: 65±3 (of a possible 70); range 58-70). All participants had given written consent prior to participation (see Appendices 1 and 2). The study was approved by the School of Psychology research ethics committee at Trinity College Dublin.

Behavioural task design

Participants were administered the TMT in the paper-and-pencil format, according to guidelines used in a standardised version of the TMT (Comprehensive Trail-Making Test, PRO-ED, Inc.). The TMT was adapted slightly by removing the last response from the end of the TMT; therefore the TMT contained the same 24 stimuli as the pcTMT. Participants completed one TMT-A and one TMT-B condition.
during the paper-and-pencil TMT. The TMT-A participants were instructed to connect the numbers sequentially, beginning at “1” and ending at “24.” The TMT-B participants were informed they needed to alternate connecting a number and then a letter in ascending order. Participants were also instructed to draw a continuous line on the paper connecting the stimuli. If a subject made a mistake during the test I, the study investigator, immediately corrected them. The participant’s scores were based on their total time to connect all 24 stimuli in the condition.

Modelling itself after the paper-and-pencil TMT, the pcTMT contained both A and B conditions but did not include the motor demand of drawing a connecting line between stimuli. To ensure that the subject was responding correctly, a simple response requirement was added for each stimulus (Figure 1). The correct response was determined by the placement of a small black square located on the outside of the circle containing the stimulus. The participants were instructed to press one of three buttons indicating if the square was on the left-side, top, or right-side of the circle containing the stimulus. When a response was made the screen was updated with a black line connecting the most recent stimuli. In this regard, the same paper-and-pencil TMT visual modality of linking stimuli was used to inform the participant of their current progress and spatial location on the screen. The task was programmed with E-Prime software v1.2 (Psychology Software Tools, Inc).

The pcTMT testing session consisted of two similar versions of the pcTMT that differed only in the total duration of the stimuli in each trial. In one version the subject was given as much time as necessary to connect all 24 stimuli in the trial. The inclusion of this untimed pcTMT afforded a direct comparison of completion times between the pcTMT and the TMT. In the second computer version, called the timed pcTMT, the participant was given 45 seconds (s) to complete as many responses as
Figure 1. Instructions for completing the pcTMT-B task. (A) Participants begin at “Start” and respond to the first stimulus (the number one) and indicate that the square is on the right side of the circle, by pressing the number three response key. (B) The participant switches from a number to a letter and responds to the second stimulus (the letter A) indicating that the square is on the top of the circle, by pressing the number two response key. Then a connecting line appears between the number one and the letter A.

possible in the trial. The rationale for the 45s fixed trial duration was to allow sufficient time to produce a robust blood oxygen level-dependent (BOLD) response and enable a block-design fMRI analysis, while still allowing for the completion of roughly the same number of responses completed in the paper-and-pencil version of the task. Each trial was preceded by a 20s baseline fixation cross. The purpose of the 20s fixation block was to allow for the hemodynamic response to return to baseline after pcTMT-A and pcTMT-B trials when used during neuroimaging, therefore decoupling any trial related residual activity. Both versions of the pcTMT contained an identical visual search for the A and B conditions. That is, the stimuli were in the same spatial locations and required the same button response decisions. Within both the timed and untimed versions, participants completed four alternating blocks of the pcTMT-A and pcTMT-B. Within the TMT and pcTMT the A condition always preceded the B condition. The order of presentation of the paper-and-pencil TMT and

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the timed and untimed versions of the pcTMT were counterbalanced across participants.

The pcTMT was presented on a 17" Sony Vaio laptop with a stimulus display size of 6.24" x 3.94." All stimuli were presented in black, Times New Roman font size 16 on a white background and a .5" black border surrounded the perimeter. During computer testing subjects were sitting directly in front of the screen, with the centre of the screen at a visual angle of 90°, and at a distance of 25" from the screen.

Statistical analysis

To examine the statistical differences that may exist between the TMT and pcTMT, a 2x2 Analysis of Variance (ANOVA) was conducted to test modality (paper-and-pencil or untimed pcTMT) by trial type (A or B). A separate 2x2 ANOVA was carried out to examine the effects of the task versions of the pcTMT (untimed or timed) by trial type (A or B). To explore the relationship between the modality (paper-and-pencil or untimed pcTMT) and trial type (A, B, difference score) Pearson’s correlation were carried out between modalities on the Trails A completion time, Trails B completion time, and difference score (Trails B – Trails A completion times). Separate analysis of the Pearson’s correlation between pcTMT test version (untimed or timed) were conducted to test the relationship between trial types (A, B, difference score) across test versions. All statistical analyses were carried out in SPSS v.11.

Results

Accuracy during the untimed pcTMT-A condition was averaged over the four trials and was 99±2% correct (Mean±SD) and during the pcTMT-B condition was 96±4% correct. Figure 2 reports the average total time (in seconds) to complete the paper-and-pencil TMT and untimed pcTMT A and B conditions. A 2x2 ANOVA of
modality (paper-and-pencil or untimed pcTMT) by trial type (A or B) was carried out. This analysis revealed a significant difference between the main effect of trial type, which showed that Trails B had a significantly longer completion time than Trails A \(F(1,19)=64.84, \ p=.0001\). However, either the main effect of modality type \(F(1,19)=1.75, \ p=.29\) nor the interaction of modality and trial type \(F(1,19)=3.83, \ p=.07\) revealed a significant difference.

![Figure 2. Total time (in seconds) to complete the TMT and untimed pcTMT. Time included for A trials, B trials, and the difference score of condition A subtracted from condition B (n=20).](image)

There was a significant Pearson’s correlation between the Trails B completion times (TMT-B: 40.9±12.8s, untimed pcTMT-B: 46.3±16.3s; \(r=.46, \ p=.04\)). Critically, there was a significant correlation between the difference scores, which was the subtraction of the Trails A from the Trails B (TMT: 13.4±12.9 s, pcTMT: 18.2±6.9 s;
There were no significant correlations between the Trails A completion times (TMT-A: 27.4±3.5s, untimed pcTMT-A: 28.1±11.2s; \( r=-.03, p=.92 \)).

Examining the pcTMT only, accuracy during the timed pcTMT-A condition averaged over the four trials was 97±6% correct and during the timed pcTMT-B condition was 94±6% correct. Figure 3 reports the average time to respond to each stimulus across the total number of responses made (in milliseconds) during the untimed and timed versions of the pcTMT. A 2x2 ANOVA of task version (untimed or timed pcTMT), by trial type (A or B), revealed a significant difference between the main effect of trial type, where Trails B took longer to complete than Trails A \([F(1,19)=169.11, p=.0001]\), and a significant interaction in which the RT in the untimed version of the task for Trails A and B was greater than the timed version \([F(1,19)=5.11, p=.04]\). No significant difference was found between the main effect of task version on RT \([F(1,19)=2.88, p=.11]\).

![Figure 3. Average response time (in milliseconds) for the untimed and timed versions of the pcTMT (n= 20).](image-url)
There were significant correlations between the timed and untimed versions of the pcTMT-A (untimed: 1172±468ms, timed: 1082±316ms; $r=.74$, $p=.0001$) and the pcTMT-B (untimed: 1930±680ms, timed: 1727±487ms; $r=.72$, $p=.0001$). Additionally, the difference scores were significantly correlated for the untimed and timed versions of the pcTMT (untimed: 758±290ms, timed: 645±239ms; $r=.66$, $p=.001$).

**Discussion**

This first experiment set out to explore the similarities between a well-established neuropsychological tool, the TMT, and a novel analogue of the TMT designed for use in the MRI scanner, the pcTMT. Two lines of investigation demonstrated that the pcTMT modality did not affect Trails B performance and also the set-shifting cost index which was derived from the difference score. First was the RT correlation investigation between the pcTMT and the paper-and-pencil version of the TMT. Significant correlations were found for both the total time to complete the B conditions and the difference scores between the B and A conditions. The difference score correlation is an important validation of the pcTMT because it is the standard scoring method of the TMT which removes individual variability in the visuomotor aspects of the task and isolates the cognitive set-shifting component. Therefore, the correlation between difference score of the original TMT and computer version showed that the inclusion of an additional response mapping technique did not interfere with the robust set-shifting demands involved during the TMT-B.

In the next analysis, the response time component of the pcTMT was investigated by comparing two versions that provided either an unlimited amount of time or 45s to complete the trial. The interaction between the two versions and the A and B trials revealed a larger switching cost in the untimed version. However, the
correlation analysis revealed that the untimed and timed versions of the pcTMT produced similar RT scores for the A and B conditions and for the difference score indicating that the timed version retained its sensitivity to the set-shifting task demands.

The TMT-A did not correlate with behavioural performance during the pcTMT-A. Although the mean reaction times were almost identical (TMT-A: 27.4s; untimed pcTMT-A: 28.1s) the greater variability contributed by outliers during the untimed pcTMT-A (S.D. 11.2s) compared to the TMT-A (S.D. 3.5s) contaminated the correlation between the modalities on Trails A. Basically, participants were getting “stuck” on one particular stimuli during the pcTMT-A, creating a longer overall task completion time, whereas in the TMT-A all stimuli took roughly the same switch time. Moreover, the pcTMT-A condition may not be an ideal neuroimaging control condition for the pcTMT-B as subjects completed a greater number of stimuli thereby making more motor responses. Consequently, a new control condition, named the pcTMT-A Paced (pcTMT-AP) task, described below, which produces an equal number of responses at a similar RT to the pcTMT-B condition, was created.

**Experiment two**

**Materials and methods**

**Participants**

Sixteen new healthy participants (9 males; Mean age: 23.4±4.2 years, range 18-34; education 16.6±2.3 years; all right-handed; all fluent English speakers) participated in the fMRI study. All participants had given written consent prior to participation (see Appendix Four). The study was approved by the School of Psychology research ethics committee at Trinity College Dublin.
fMRI task design

The new control condition, the pcTMT-AP, required the subject to perform the same, which was to locate the number and respond task as the original pcTMT-A. However, a pre-calculated delay (e.g. The result of the pcTMT-B mean RT minus pcTMT-A mean RT) was imposed between responses to stimuli thereby requiring the participant to wait before they could respond to the next stimulus in the sequence. The fMRI pcTMT task participants first completed one trial of the timed pcTMT-A (same task used in the behavioural study), followed by a trial of the timed pcTMT-B. From these trials the average time to respond to each stimulus was calculated for both conditions. The A condition average RT was then subtracted from the B condition average RT to obtain the difference time between conditions and this served as the imposed delay between stimuli on the pcTMT-AP. The inclusion of these individually tailored wait times allowed us to equate the number of responses made in the A and B trials. Figure 4 illustrates the pcTMT-AP, depicting how the delay time between the presentation of the stimuli required the participants to wait to make their next response. This wait period, in which all stimuli were removed and only previous responses and empty circles remained, controlled for the number of responses and button press decisions the participants made during an AP trial. Once the waiting period was complete all stimuli would reappear and participants would find and respond to the next stimulus. Additionally, the stimuli in both the AP and B trials were in the same spatial location and had the black square in the same positions, which meant the same button decisions were made in the same order in the two conditions.

The trials were also programmed to account for differences in the number of responses made by each participant. If the participant completed 24 responses before
the end of the 45 s response period a fixation cross would appear for the remainder of the time. Repressors in the fMRI block design analysis were tailored to reflect these individual durations.

Figure 4. Instructions for completing the pcTMT-AP. (A) Participant begins with a screen containing empty circles. Participant waits for a pre-programmed amount of time. (B) The stimuli appear after the wait time is over. Participants begin at “Start” and respond to the first stimulus (the number one) and indicate that the square is on the right side of the circle, by pressing the number three response key. (C) The previous correct stimulus is left on screen while the other stimuli are removed. Participant waits for pre-programmed amount of time until the stimuli reappear. (D) All of the stimuli reappear in the same positions after the wait time is over. Participant locates the next sequential number and responds to the second stimulus (the number two) indicating that the square is on the left side of the circle, by pressing the number one response key. (E) The previous correct stimuli are left on the screen and a connecting line appears.

Procedure

All participants were given instructions outside of the scanner before the start of imaging. They were given practice blocks of the pcTMT-AP and pcTMT-B, in which they had to reach an accuracy score of 90% correct on each condition before they were allowed to proceed to the scanning session (Note: Only three participants...
needed one extra practice block of the B trials to reach the 90% accuracy cut-off threshold.) Additionally, participants completed the paper-and-pencil version of the TMT. During the scanning session, participants completed nine trials, which consisted of one block of pcTMT-A trials, and then alternated between four blocks of pcTMT-B trials and four blocks of pcTMT-AP trials.

**Imaging acquisition**

Each participant underwent a single fMRI scanning session. All scanning was acquired on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a 30.5 cm internal diameter three-axis local gradient coil with insertable radiofrequency coils with transmit–receive capabilities. Participants were instructed to lay supine on the scanner bed and an RF head coil was positioned over their head and was equipped with a mirror affixed to it which permitted them to view stimuli projected onto a screen at the top of the scanner bed. To minimize head movement and artefact in the coil during data acquisition, subject’s heads were cushioned with padding.

Each scanning sequence began with a reference scan to resolve sensitivity variations. A parallel Sensitivity Encoding (SENSE) approach (Pruessmann, Weiger, Scheidegger, & Boesiger, 1999) with a reduction factor of two was utilized for all T1-weighted image acquisitions. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV 230 mm, thickness 0.9 mm, voxel size 0.9 mm × 0.9 mm × 0.9 mm) were acquired (total duration 325s) prior to the functional scans. The anatomical scan allowed for subsequent activation localization and spatial normalisation.

Functional data were collected using a T2* weighted echo planar imaging (EPI) sequence that acquired 32 non-contiguous (10% gap) 3 mm axial slices covering the entire brain (TE=35ms, TR=2000ms, FOV 224 mm, 64 mm × 64 mm
matrix size in Fourier space) covering the whole brain. At the beginning of each of the nine functional runs four discarded acquisition scans (8s) were taken to allow for the stabilisation of T1 effects on the BOLD signal.

fMRI analysis

Functional data were processed with Analysis of Functional NeuroImages (AFNI) software (Cox, 1996). Following image reconstruction, the eight 3D time series (runs two through nine) were concatenated and motion-corrected using 3-D volume registration (least-squares alignment of three translational and three rotational parameters). Activation falling near the outermost surface of the brain was removed using edge detection techniques.

A block design analysis calculated the percentage change in activation between the task active periods (pcTMT-B or pcTMT-AP) and the rest period baselines, which was 20s of a fixation cross. These On-Off regressors (i.e. pcTMT-B vs. Rest and pcTMT-AP vs. Rest) were tailored for each subject to reflect the actual durations of the conditions and were convolved with a standard hemodynamic response to accommodate the lag time of the BOLD response.

The percentage change activation maps were resampled to 1 mm x 1 mm x 1 mm resolution, then normalised through warping the images into standard Talairach space (Talairach & Tournoux, 1988) and then spatially blurred with the nearest neighbour with a 4.2 mm isotropic rms Gaussian kernel. Group activation maps were then created for each condition (pcTMT-B and pcTMT-AP) and were determined with one-sample t-tests against the null hypothesis of zero activation change. Significant voxels passed a voxelwise statistical threshold \[t(15)=4.073, p<.001\] and were required to be part of a larger 232 µl (microlitres) cluster of contiguous significant voxels. Cluster size criterion thresholding, to correct for multiple
comparisons, was determined using the AlphaSim program in the AFNI toolbox which ran 1,000 Monte Carlo simulations at p=.001 and resulted in a 5% probability that a cluster would survive due to chance.

To further compare activations between the B and AP conditions a whole-brain, voxelwise, two-factor mixed effects ANOVA was conducted to explore the mean percentage change from baseline across the brain for each condition in each participant. Included in the ANOVA were the two fixed factors, which were the task conditions pcTMT-B and pcTMT-AP, and the random factors were the 16 participants. Significant voxels passed a voxelwise threshold \([F(1,15)=10.798, \ p<.005]\) and were required to be part of a 278\mu l contiguous cluster, resulting in a 5% probability of a false positive cluster.

**Results**

**fMRI task performance**

Mean accuracy for the pcTMT-B was 97±16% correct and during the AP trials was 97±6%. The timing manipulation to equate numbers of responses made during the A (20.6±5.2 responses) and B (20.6±5.2 responses) trials proved effective with no significant difference found. Subjects completed the paper-and-pencil TMT before scanning. A 2x2 ANOVA of modality (paper-and-pencil or pcTMT), by trial type (A or B) revealed a significant difference between trial type \([F(1,15)=77.51, \ p=.0001]\) and modality \([F(1,15)=7.35, \ p=.02]\). However, the critical interaction between trial type and modality \([F(1,15)=1.94, \ p=.18]\) was not significantly different, revealing that trial type differences were not effected by the modality.

There were strong correlations between the A switch times (TMT-A: 1541±661ms, pcTMT-AP: 1215±539ms; \(r=.69, \ p=.01\)) and between the B switch times (TMT-B: 2146±772ms, pcTMT-B: 2009±849ms; \(r=.89, \ p=.0001\)). Yet, there
was no correlation between the difference scores, which was the subtraction of the Trails A from the Trails B (TMT: 604±434ms, pcTMT: 794±401ms; r=.15, p=.57), which is most likely due to the different method of subtraction used in the paper-and-pencil and fMRI modalities.

**fMRI activation**

The activation map for the AP and B conditions contained wide-spread activity in frontal, parietal, and temporal regions. Figure 5 shows these extensive regions of activation during the B condition (p<.001, uncorrected). The voxelwise ANOVA (p<.005, corrected) between conditions identified four areas of significant difference (Table 1).

The pcTMT-B condition had greater activity than pcTMT-AP in three regions including right inferior and middle frontal gyri \( F(1,15)=6.14, p=.0001 \), right precentral gyrus \( F(1,15)=4.13, p=.001 \), and a region encompassing containing left angular gyrus and left middle temporal gyrus \( F(1,15)=4.83, p=.001 \) (Figure 6A-C). The right fusiform gyrus was the only region more active during the pcTMT-AP condition \( F(1,15)=-5.4, p=.0001 \) (Figure 6D).

**Table 1**
**Activation differences between the pcTMT-B and AP conditions.** These clusters were derived from whole-brain, voxelwise two-factor mixed effects ANOVA (p<0.005, corrected). The analysis explored the differences in mean percentage change, which was relative to the rest period, between the pcTMT-B and pcTMT-AP conditions.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Side</th>
<th>BA</th>
<th>(µl)</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusiform gyrus</td>
<td>R</td>
<td>19</td>
<td>705</td>
<td>38</td>
<td>-66</td>
<td>-7</td>
<td>A&gt;B</td>
</tr>
<tr>
<td>Inferior/middle frontal gyrri</td>
<td>R</td>
<td>47</td>
<td>443</td>
<td>36</td>
<td>34</td>
<td>-3</td>
<td>B&gt;A</td>
</tr>
<tr>
<td>Precentral gyrus</td>
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<td>375</td>
<td>31</td>
<td>-1</td>
<td>31</td>
<td>B&gt;A</td>
</tr>
<tr>
<td>Middle temporal/angular gyrri</td>
<td>L</td>
<td>39</td>
<td>365</td>
<td>-35</td>
<td>-68</td>
<td>28</td>
<td>B&gt;A</td>
</tr>
</tbody>
</table>
Figure 5. Activation map of the pcTMT-B condition vs. Rest. Axial images reveal extensive activity in frontal, parietal, and temporal cortices, during the pcTMT-B condition (p<0.001, uncorrected).

Discussion

The aim of this study was to develop an analogue of the well-known Trail Making Test, named the pcTMT, which would be suitable for fMRI and thus allow us to identify brain regions involved during this demanding set-shifting task. This investigation revealed set-shifting to be associated with the right inferior and middle frontal gyri, right precentral gyrus, and left middle temporal and angular gyri. We attribute the greater activation in the fusiform gyrus during the A control condition to the different presentation of stimuli during the AP task. The AP task was designed to
Figure 6. Significant areas of cortical activity from the whole brain, voxelwise, two-way ANOVA displaying the contrast between pcTMT-B vs. pcTMT-AP conditions ($p<.005$, corrected). The three regions that were more active during the B condition included: (A) right inferior and middle frontal gyri (yellow), (B) right precentral gyrus (blue) and (C) left middle temporal and angular gyri (green). (D) The one AP region that was more active during the A control condition is d. right fusiform gyrus (red).
slow down the participant’s responses to match the frequency of responding on the B trials (this change may also be responsible for the lack of correlation in the B minus A difference score between the paper-and-pencil version of the TMT and the imaged version of the pcTMT). Slowing the participant’s responding involved the temporary removal of all stimuli from the screen and subsequently created a flicker of the stimulus which likely caused the increased fusiform activation. Our fusiform region of activation encompassed the V5/MT area of the visual cortex and the temporal modulation (flicker) of a stimulus has been shown to elicit a response within V5 (Tootell, et al., 1995). Additionally, the fusiform gyrus is an area highly responsive to the recognition of numbers (Allison, McCarthy, Nobre, Puce, & Belger, 1994), which would occur more frequently during the numbers-only A condition.

Another goal in developing the pcTMT was to create a task that contained no potentially confounding differences between the A or B conditions. Gaudino and colleagues (1995) have found that the TMT-A and B differ in perceptual and motor demands. For example, they found the B condition to require more time to complete due to the larger distances between successive stimuli relative to the A condition. Their work lead researchers to question whether the TMT was reflecting deficits in set-shifting or in visuomotor Sequencing. In the D-KEFS neuropsychological battery (McDonald, Delis, Norman, Tecoma, & Irugu-Madozi, 2005) their TMT sub-test attempted to parse out the component processes involved in set-switching and included four baseline conditions (visual scanning, number sequencing, letter sequencing, and motor speed). In a study of patients with focal lesions in the lateral prefrontal cortex that utilized the D-KEFS TMT they found patients were only impaired on the number-letter switching test and not on any of the four baseline measures. The pcTMT employed here presented equal numbers of stimuli in the same
spatial locations in the A and B conditions. This, in turn, engaged the same visual and motor demands in each condition, thereby isolating the switching component when the A condition was subtracted from the B condition.

**Components of set-shifting**

A high correlation between the B condition of the paper-and-pencil TMT and pcTMT was found. I attribute this finding to the stability of the set-shifting components engaged during the Trails B task, regardless of the response modality, that is, whether responses were made by drawing a line with a paper and pencil or pressing a button on a computer keyboard. Set-shifting occurring during Trails B requires executive functioning that is facilitated by an interaction of higher order cognitive processes. During this cognitive processing a "procedural schema" is created to describe how one must follow an appropriate plan of action to control the interaction of mental abilities initiated by an exogenously generated event and thus requiring us to implement and monitor the plan, making adjustments as necessary (Norman & Shallice, 1986). One of the processes required to carry out a goal-directed cognitive shift is working memory. Neuroimaging studies indicate that storage of information during working memory occurs in the posterior region of the brain (mainly the parietal lobe) and the maintenance and manipulation of information occurs in the front of the brain (mostly prefrontal cortex). The set-shifting properties of the TMT have also served as a proxy measure to underscore frontal lobe functioning (Hänninen, et al., 1997). Left DLPFC has been shown to reflect the cognitive set-shifting that occurs during fMRI studies of the TMT (Moll, de Oliveira-Souza, Moll, Bramati, & Andreiuolo, 2002; Zakzanis, Mraz, & Graham, 2005). Although I did not find left DLPFC activity at our voxelwise threshold of $p=0.005$, 322
when a more liberal threshold of $p=.01$ was used extensive left DLPFC activation was observed.

**Brain regions involved in cognitive set-shifting during the pcTMT**

The present study identified set-shifting with the right inferior and middle frontal gyrus, the right precentral gyrus and a left hemisphere temporo-parietal region. Right lateralised prefrontal activation has been consistently found in the neuroimaging literature to be specialised for retrieval during working memory tasks (see review in Tulving, Kapur, Craik, Moscovitch, & Houle, 1994) engaging perceptual and visuospatial processes (Lie, Specht, Marshall, & Fink, 2006), and response suppression during GO/NOGO tasks (Garavan, et al., 1999). The ability to correctly switch from counting numbers to completing the alphabet relies heavily on the ability to perceive and continuously retrieve the correct response when having to switch from one set of stimuli to the other.

Neuroimaging studies have shown the right inferior frontal gyrus (IFG) to be engaged during the set-shifting Wisconsin Card Sorting Task (Konishi, et al., 1998) (Berman, et al., 1995). The right IFG is intimately involved in inhibitory control processes (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Garavan, et al., 1999) and in tasks involving set switching (Brass, et al., 2003; Dreher & Berman, 2002). Furthermore, right frontal cortex lesion patients, particularly those with damage to the pars opercularis (POp) (Casey, et al., 1997), have shown high correlations with poorer set-shifting abilities. Damage to the POp disrupts the ability to activate inhibition mechanisms responsible for suppression of incorrect responses during a task switch (Aron, Monsell, Sahakian, & Robbins, 2004). This inability to inhibit can be explained by the fact that the IFG is one of the most well-connected regions in the PFC (Miller & Cohen, 2001) including important links to the
subthalamic nuclei shown to be important for motor inhibition (Aron & Poldrack, 2006).

The pcTMT also revealed significant activation during the B condition in the precentral gyrus. Located in the dorsal part of the primary motor cortex, the precentral gyrus is an integral component involved in the maintenance phase of working memory (Tsukiura, et al., 2001; Zarahn, Rakitin, Abela, Flynn, & Stern, 2005). Although the precentral gyrus is not as highly specialized in top-down control as is, for instance, the DLPFC, it may play an active role in maintaining a task set while performing the switch. The left temporo-parietal activation, which has previously been observed for TMT-B trials (Zakzanis, Mraz, & Graham, 2005) may reflect working memory demands in switching from numbers to letters during the TMT-B. When examining a clinical population, poor performance on Trails B among schizophrenia patients has been attributed to an impairment in working memory (Gold, Carpenter, Randolph, Goldberg, & Weinberger, 1997); positing that schizophrenia is related to a difficulty in maintaining task context information in memory (Meiran, Levine, & Henik, 2000). Furthermore, impairments in thought disturbance and disorganisation were significant predictors of poor set-shifting performance in patients (Pantelis, et al., 2004). Therefore, the pcTMT can potentially be used as a tool to link together clinical symptomology and dysfunctional brain activity. This can be done utilizing the pcTMT by examining behavioural deficits in speeded processing and maintaining attention during goal-directed behaviours, which have been localized in the PFC, motor, and temporo-parietal recruitment.

**Conclusion**

Although the pcTMT does show future utility in the usage of assessing cognitive functioning in clinical populations further testing needs to be carried out
first behaviourally in these clinical groups to ensure performance differences exist on this version of the TMT. Furthermore, an inherent limitation of cognitive activation paradigms reveals that localizing set-shifting dysfunctions during neuroimaging only allows for a glimpse at disrupted brain activity and does not convey the entire network of activity that underlies a complex clinical illness, such as schizophrenia.

In regards to task design, the fixation blocks used in the pcTMT did not allow for engagement of an active baseline task. In future testing of the pcTMT a fixation condition which controls for eye movement and motor responding will be used to better serve as a contrast from task active trials. The lack of correlation found between the Trails A paper-and-pencil TMT and pcTMT versions needs to be address in the creation of a new control condition for Trails B which allows for correlations between Trails A on the two modalities.

Future testing of the pcTMT needs to be completed to ensure strong correlations between the original task version and the computerized version exist. The imaging results show great promise in localising in set-shifting abilities, which are known to be impaired in schizophrenia, and can hopefully be used in identifying the neural mechanisms contributing to the cognitive deficits this illness.
Appendix Two

pcTMT behavioural study: Consent form

Title of Project:

Behavioural investigation of cognitive control functions

Research Team:

Dr. Hugh Caravan, Trinity College Dublin
Sarah Jacobson, Trinity College Dublin

Information about the Project:

In this study we are seeking to identify the brain regions involved in “executive” or cognitive functions. Executive functions are the skills involved in controlling behaviour. Examples of executive functions include the ability to stop a response that you have already started, the ability to perform multiple tasks at the same time and to monitor your behaviour to detect how well you are doing on a task. To identify the brain regions involved in these cognitive abilities, you will be asked to perform some behavioural tasks.

Task Description
You will be asked to complete a task that measures attention and other cognitive functions. You will be given the instructions on how to complete the task then asked to do a practice on a computer to show that you understand the task. You will then be asked to complete a computer version of the task and a paper and pencil version as well.

The study will take place at the Lloyd Institute located at Trinity College Dublin. The session will take approximately 1 hour. For participating in the study, you will receive either Psychology undergraduate research credits or cash at 10 euros per hour. If at any time during the research you wish to withdraw from the study then you may. All information gathered during the course of this research is available to you upon request, and will be held in the strictest of confidence. Please ask the researcher any questions you may have. If you are happy to be involved in this study, then please complete this consent form and make it available to the researcher(s).

In the instance that you should feel fatigued or restless during the behavioural testing feel free to ask the researcher(s) for a break at any time.

If, at any time, you have any questions regarding this research, please feel free to contact Dr Hugh Caravan on (01) 8968448 or Sarah Jacobson on (01) 8968417. You can also feel free to contact them at:
Trinity College Institute of Neuroscience
Lloyd Institute
Trinity College
Dublin 2
By providing my consent I agree that:

I have been informed of the discomforts and risks that I may reasonably expect to experience as part of this study. I have been informed that I will be asked to perform some tasks that I have been trained on, which should not cause undue distress.

I understand these risks and am agreeing to volunteer to participate in this research. I understand that I can withdraw at any time from the study.

PARTICIPANT'S NAME: ____________________________________________

PARTICIPANT'S SIGNATURE: _______________________________________

Date: ____________________

WITNESS'S NAME: _______________________________________________

WITNESS'S SIGNATURE: _________________________________________

Date: ____________________
### Appendix Three

**All MRI studies: TCIN MRI consent form**

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<thead>
<tr>
<th>Name:</th>
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<tbody>
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<td>Phone-</td>
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</tr>
<tr>
<td>Sex:</td>
<td>Male [ ] Female [ ]</td>
</tr>
<tr>
<td>Date of Birth:</td>
<td></td>
</tr>
<tr>
<td>Weight:</td>
<td>kg</td>
</tr>
</tbody>
</table>

Please provide us with the details of another person (e.g., next-of-kin) should we need to contact you in the future.

| Name of contact person: |  |
| Phone: |  |

Please provide us with the details of your GP should we need to contact him/her in the future.

| Name of GP: |  |
| Phone: |  |
| Address: |  |

| Study: |  |
| Time in: | : | Time Out: | : |
| Investigator: |  |
Information:
Your MR study

As part of your study we will obtain a limited number of pictures of your brain. Our research studies are designed to improve our knowledge of the brain. They are not designed for diagnostic or clinical purposes.

Minor changes are sometimes found in completely healthy people. You should be aware that because our pictures are taken for a specific research purpose, not all abnormalities that might be detected by other MR scans are necessarily seen. On extremely rare occasions, we might find an abnormality that is significant and which should be investigated further. If we find such a significant abnormality in your brain, we will contact you directly and will likely recommend that you follow up these findings by contacting your own doctor.

Although a significant abnormality is extremely unlikely, you should be aware that if such an abnormality is detected and you are informed, then this knowledge may have consequences for you. Please take the time to consider carefully what it would mean to you if we told you of an abnormality in your brain which might, or might not, affect you later in life. Knowledge of an abnormality may affect your ability to work in certain professions, obtain life or health insurance and other facets of daily living. If you do not want to know, then it is better not to participate.

There are some items that may interfere with the Magnetic Resonance Imaging and some that may be potentially hazardous. To help us to determine your suitability for an MRI scan and to ensure your safety, please complete the following checklist carefully.

Not all people can have an MRI scan because the strong magnetic field may be hazardous to them.

- People with
  - permanent pacemakers
  - prosthetic heart valves
  - implanted cardiac defibrillators
  - certain types of vascular clips

  Cannot have an MRI

Instructions for Patient/Volunteers

1. You are urged to use the earplugs or headphones we supply during your MRI examination since some patients may find the noise levels unacceptable, and the noise levels may affect your hearing.
2. Remove all jewelery (eg, necklaces, pins, rings).
3. Remove all hairpins, bobby pins, barrettes, clips, etc.
4. Remove all dentures, false teeth, and partial dental plates.
5. Remove hearing aides.
6. Remove eyeglasses.
7. Remove your watch, pager, cell phone, credit cards, bankcards, and all other cards with a magnetic strip.
8. Remove body piercing objects.
9. Use gown, if provided, or remove all clothing with metal fasteners, zippers, etc.

CHECK LIST FOR 3T MAGNETIC RESONANCE IMAGING

Do you have any of the following:

<table>
<thead>
<tr>
<th>Cardiac Pacemaker:</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever had any surgical procedures?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>If yes, what type and where</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following items may be harmful to you during your MR scan or may interfere with the MR examination. You must provide a “yes” or “no” for every item. Please indicate if you have or have had any of the following:

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Any type of electronic, mechanical, or magnetic implant</th>
<th>Type:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cardiac pacemaker</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aneurysm clip</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Implanted cardiac defibrillator</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neurostimulator</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biostimulator</td>
<td>Type:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any type of internal electrodes or wires</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cochlear implant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hearing aid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Implanted drug pump (e.g., insulin, Baclofen, chemotherapy, pain medicine)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Halo vest</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinal fixation device</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinal fusion procedure</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any type of coil, filter, or stent</td>
<td>Type:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any type of metal object (e.g., shrapnel, bullet, BB)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Artificial heart valve</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any type of ear implant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penile implant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Artificial eye</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eyelid spring</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Any type of implant held in place by a magnet</td>
<td>Type:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Any type of surgical clip or staple</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Any IV access port (e.g., Broviac, Port-a-Cath, Hickman, Picc line)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Medication patch (e.g., nitroglycerine, nicotine)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Shunt</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Artificial limb or joint</td>
<td>What and where:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tissue expander (e.g., breast)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Removable dentures, false teeth, or partial plate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diaphragm, IUD, Pessary</td>
<td>Type:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surgical mesh</td>
<td>Location:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Body piercing</td>
<td>Location:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wig, hair implants</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tattoos or tattooed eyeliner</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Claustrophobia</td>
<td></td>
</tr>
</tbody>
</table>

Handedness: Right / Left
I have read and understood this form, and consent to my study being used for research. I have had a chance to ask any questions.

My contact phone number is: ______________________________________

My contact address is: ____________________________________________

______________________________________________________________

Signed (Volunteer): _____________________________________________

Parent or Guardian if under 18: ___________________________________

Witnessed (Researcher/MR staff): _________________________________

Date: __________________________________________________________

Hazard Checklist for MRI Personnel

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>_____</td>
<td>Endotracheal tube</td>
</tr>
<tr>
<td>_____</td>
<td>Swan-ganz catheter</td>
</tr>
<tr>
<td>_____</td>
<td>Extra ventricular device</td>
</tr>
<tr>
<td>_____</td>
<td>Arterial line transducer</td>
</tr>
<tr>
<td>_____</td>
<td>Foley catheter with temperature sensor and/or metal clamp</td>
</tr>
<tr>
<td>_____</td>
<td>Rectal probe</td>
</tr>
<tr>
<td>_____</td>
<td>Esophageal probe</td>
</tr>
<tr>
<td>_____</td>
<td>Tracheotomy tube</td>
</tr>
<tr>
<td>_____</td>
<td>Guidewires</td>
</tr>
</tbody>
</table>

Comments:
GENERAL MRI DATA CONSENT FORM

Trinity College Institute of Neuroscience, (TCIN) is performing research, utilising an MRI scanner at Trinity College, Dublin 2. These research scans, although not full clinical scans, will be read by a clinician.

In the event of an irregularity being found, the clinician, [Dr William Torreggiani of The Adelaide and Meath Hospital Incorporating the National Children's Hospital (AMNCH), Tallaght] will inform the participants GP, that a proper clinical scan may be required to determine whether or not an irregularity is of clinical significance.

To enable us to perform the research scans the participant agrees to give consent/ permission for:

(i) TCIN to conduct the MRI scan and take MRI scan data of participant;
(ii) TCIN or PI to contact participants GP;
(iii) TCIN radiographer to send MRI scan data to radiologist acting for TCIN;
(iv) Radiologist to store data in a hospital system with same care as other patient data;
(v) Radiologist/ Clinician (acting for TCIN) to contact participants GP;
(vi) TCIN to store data on the study for a period of at least 5 years.

A dated standard letter signed by the appropriate PI will be sent to all participants GP’s, it is the responsibility of the PI to ensure that this is sent at least two days before scanning to allow for postal delays. The principal investigator is responsible for their project at all times.

The clinician will be sent data in a form that allows identification so that if a response is required he can act quickly. This will be stored in the hospital system and treated like other medical data, as per the rules of that institution. The raw scan data will be stored at TCIN in anonymous form for research purposes as agreed on the consent form of the particular research project.

I agree to the above points and understand that my data will be treated carefully at TCIN and in the hospital system.

Participant Name and Address___________________________________________

Signed by Participant:______________________________________________

Participants GP Name and Address

______________________________________________

Date: ________________________________________
Appendix Four

pcTMT fMRI study: Consent form

Title of Project:
FMRI investigations of cognitive control functions

Research Team:
Dr. Hugh Caravan, Trinity College Dublin; Sarah Jacobson, Trinity College Dublin

Information about the Project:

In this study we are seeking to identify the brain regions involved in “executive” or cognitive functions. Executive functions are the skills involved in controlling behaviour. Examples of executive functions include the ability to stop a response that you have already started, the ability to perform multiple tasks at the same time and to monitor your behaviour to detect how well you are doing on a task. To identify the brain regions involved in these cognitive abilities, you will be asked to perform some tasks while we image your brain using Magnetic Resonance Imaging (described below). Prior to the imaging, you will be asked to practice the tasks so you will know what to do once in the scanner.

Task Description
You will be asked to complete a task that measures attention and other cognitive functions. You will be given the information on how to complete the task then asked to do a practice on a computer to show that you understand the task. You will then do the same task while in the scanner. You will see the task presented on a screen and the instructor will show you how to respond. This task should take no more than 10 minutes to learn and will take less than 20 minutes to complete in the scanner.

The study will take place at the MRI facility located at Trinity College Dublin. The session (including the MRI scanning) will take between 1.5 and 2.5 hours. For participating in the study, you will receive either Psychology undergraduate research credits or cash at 10 euros per hour (we will round up to the nearest hour so a 2.5 hour study will compensate you with 30 euros). If at any time during the research you wish to withdraw from the study then you may. All information gathered during the course of this research is available to you upon request, and will be held in the strictest of confidence. Please ask the researcher any questions you may have. If you are happy to be involved in this study, then please complete this consent form and make it available to the researcher(s).

If, at any time, you have any questions regarding this research, please feel free to contact Dr Hugh Caravan on 01 608 8448/01 608 3910.
What is MRI?

The purpose of functional MRI scanning is to determine which brain regions are activated as someone performs certain tasks. In the MRI scanner there is a very large magnetic field. This magnetic field and radio signals which are transmitted in the scanner measure the concentration of water particles within the body, allowing brain functioning relating to behaviour to be measured in terms of blood flow to the brain. The person who is going to be scanned lies on a bed where their head is placed into a device which has the appearance of a large helmet. When the person has been safely and comfortably secured in this device, the bed is moved slowly into the scanner. When the person’s head is in the middle of the magnetic field, radio frequency pulses and magnetic fields are switched on and off to produce a signal which we use for measuring blood flow.

Individual MRI test runs will last no longer than 10 minutes to minimise fatigue and the entire testing session will be completed within 60 minutes. It is very important that you keep still and, in particular, do not move your head while we are taking an image of your brain. For some images, you will be doing a cognitive task that you will have practiced outside of the scanner. For other images, you will just lie still and relax while we take high-resolution images of your brain. We will explain exactly what you need to do before we start each MRI test run.

What will I be asked to do while I am in the MRI scanner?

You will be asked to perform cognitive tasks that you will have already practiced with one of the researchers prior to the scanning session. During scanning, we will tell you what task to do before each scan by communicating through the intercom system.

What are the risks associated with MRI?

When operated by appropriately qualified individuals, MRI presents virtually no risk, as there is NO exposure to x-rays or radioactivity with this procedure. The noise produced by an MRI exam can be very loud and you will be issued with protective headphones or earplugs to prevent damage to your hearing. The noise produced by the exam has been reported to produce temporary threshold shifts (i.e., decreased ability to hear quiet sounds) in a small percentage of people. Given the confines of an MRI machine, a small percentage of people in the past have reported feeling claustrophobic (fear of being closed in a tight space) when placed into an MRI scanner. Please let us know if you have experienced claustrophobia in the past. During MRI scanning, you will be in contact with the MRI operator via an auditory communication system. This will be used to regularly check your comfort and to allow you to inform us of any problems or concerns. You will also have a "panic button" which you may press at any time to indicate that you wish to stop the scanning procedure.
As the MRI involves a large magnetic field, it is essential that **NO METAL BE BROUGHT INTO THE SCANNER WITH YOU.**

Items that **must be** removed by individuals before entering the MRI facility include:

- Purse, wallet, money clip, credit cards, cards with magnetic strips;
- Electronic devices such as beepers or cell phones;
- Hearing aids;
- Metal jewelry (in all parts of the body), watches;
- Pens, paper clips, keys, coins;
- Hair barrettes, hairpins;
- Any article of clothing that has a metal zipper, buttons, snaps, hooks, underwire bras, or metal threads;
- Shoes, belt buckles, safety pins.

Other objects that may be hazardous include:

- Metallic spinal rod
- Plates, pins, screws, or metal mesh used to repair a bone or joint
- Joint replacement or prosthesis
- Metal jewelry such as that used with body piercing.
- Some tattoos or tattooed eyeliner (these alter MR images, and there is a chance of skin irritation or swelling; black and blue pigments are the most troublesome)
- Bullet, shrapnel, or other type of metal fragment
- Metallic foreign body within or near the eye (such an object generally can be seen on an x-ray; metal workers are most likely to have this problem)
- Dental fillings (while usually unaffected by the magnetic field, they may distort images of the facial area or brain; the same is true for orthodontic braces and retainers)

**If you have any of these items, please inform us immediately.**

There may be additional or unknown risks associated with MRI. For example, in very rare cases, the strong magnetic field can induce nerve stimulation (e.g., switching the strong magnetic field gradients during imaging has been reported to cause twitching in the neck muscles). Also, in very rare cases, the radio signals have been reported to cause burns. There may be other risks associated with imaging that are not yet known.

**Who shouldn't undergo the MRI procedure?**

Research participants who have the following items **should not** undergo an MRI procedure:

- Cardiac pacemaker or an implanted defibrillator
- Catheter that has metal components that may pose a risk of a burn injury
- A metal clip placed to prevent bleeding from an intra-cranial aneurysm
• A medication pump (such as that used to deliver insulin or a pain-relieving drug)
• A cochlear (inner ear) implant

It is essential that you inform the MR operator if you have any metal items in any of the above lists.

Pregnancy and MRI

For female participants it is also important that you tell us if there is any possibility that you are pregnant. To date there are no known risks of MRI during pregnancy, however as a precautionary safety measure pregnant individuals will not be included in the study. To participate in the current study women of child-bearing potential must be using one of the following acceptable methods of birth-control:

a. oral or transdermal contraceptives
b. barrier (diaphragm or condom) with spermicide
c. intrauterine progesterone contraceptive system
d. levonorgestrel implant
e. medroxyprogesterone acetate contraceptive injection
f. complete abstinence from sexual activity

What if the brain imaging finds some abnormality in my brain?

If a brain abnormality is observed by a researcher, then our procedure will be to first obtain a reading by an MRI radiologist. Radiologists will be available as there will be a part-time clinical service provided at the Neuroscience Institute. If the radiologists suggests that the abnormality requires follow-up then we will contact the participant and request a face-to-face meeting. Every effort will be made to ensure that the radiologist is present for this meeting – if not, the meeting will be held in the Psychology department where there are clinical psychologists and counsellors available and one will be asked by the researcher to be present or to be available.

Every effort will be made to communicate to the participant the necessity to follow-up on our observation while at the same time attempting to minimise alarm. We will make it clear to the participant that our images are not clinically-prescribed but are for the purposes of research. Consequently, they may not be of an adequate quality for clinical diagnosis. We will also request contact details of the participant’s GP and will ask the participant’s permission to contact the GP directly.

By providing my consent I agree that:

I have been informed of the discomforts and risks that I may reasonably expect to experience as part of this study. I have been informed that if a brain abnormality is
observed, that I will be contacted for a meeting with a radiologist. I have been informed that when used on appropriately qualified individuals, MRI presents virtually no risk. There will be no exposure to x-rays or radioactivity in this study. I understand that noise produced by this exam could be very loud, and that I will wear earplugs or headphones to prevent damage to my hearing. Even with earplugs, the noise produced by the exam may produce temporary threshold shifts (i.e., decreased ability to hear quiet sounds). I have been informed that I may experience some discomfort from lying in the MRI scanner such as claustrophobia (fear of being closed in a tight space) or tight sensations from having my head restrained to prevent movement. I have been informed that I will also be asked to perform some tasks that I have been trained on, prior to the MRI procedure, which should not cause undue distress.

I have been informed that other risks of injury due to MRI include damage to implanted electronic devices (such as pacemakers), haemorrhage if aneurysm clips are present and trauma if ferrous metal objects are brought too close to the scanner. However, these risks are minimal in a properly administered site. I do not have any of these items in my body.

I understand these risks and am agreeing to volunteer to participate in this research. I understand that I can withdraw at any time from the study.

PARTICIPANT'S NAME: ______________________________

PARTICIPANT'S SIGNATURE: ______________________________

Date: __________________

WITNESS'S NAME: ______________________________

WITNESS'S SIGNATURE: ______________________________

Date: __________________
Appendix Five

Receptive language, motor and executive deficits in adolescents with subclinical psychotic symptoms

Acknowledgement of journal submission for this appendix:

* Both authors contributed equally to this work

* Both authors contributed equally to this work

^aDepartment of Psychiatry, Royal College of Surgeons Ireland, Dublin, Ireland, ^bSchool of Psychology and Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland; ^cDepartment of Psychiatry, Beaumont Hospital, Dublin, Ireland
Abstract

We examined neuropsychological functioning at age 11-13 in adolescents who reported subclinical psychotic symptoms on interview and adolescents who did not report such symptoms. Participants were recruited from local primary schools. We found that adolescents who report subclinical psychotic symptoms exhibit a subtle but significant pattern of impairments in receptive language, motor function and executive function (set-shifting) compared with adolescents without such symptoms. Taken together with the results from birth cohort, genetic high risk and prodromal studies, these findings suggest a developmental trajectory of neural compromise which can give clues to the cognitive processes and brain regions earliest-affected in psychosis.
Introduction

Population-based studies show that about 5% of adults report subclinical psychotic symptoms.\(^1\) The prevalence of such symptoms among adolescents on interview is 13-15% \(^2,3\) and is even higher when using questionnaire-based responses.\(^4\) The importance of subclinical symptoms lies in the possibility that such symptoms increase the risk of later psychotic illness in adulthood.\(^2\) Adolescents with subclinical symptoms could therefore be conceptualised as a symptomatic high-risk group for later psychotic disorder, (in the same way as offspring of parents with schizophrenia comprise a genetic high risk group), and may yield insights into the earliest pathological processes in the trajectory to adult psychosis. The present study investigated neurocognitive functioning in relation to subclinical psychotic symptoms in adolescents aged 11-13 years old.

Materials and Methods

Participants

Thirty-seven adolescents aged 11-13 attended for neuropsychological testing in Beaumont Hospital, Dublin. These comprised 17 adolescents with subclinical psychotic symptoms (7 male and 10 female, mean age 11.6 years) and 20 children without such symptoms (5 male and 15 female, mean age 12.1 years). Subjects had been recruited from local primary schools following administration of a screening questionnaire. The presence of symptoms was verified by clinical interview. The interview schedule used in this study was the Schedule for Affective Disorders and Schizophrenia for School-aged Children, Present and Lifetime Versions (K-SADS-PL).\(^5\) Interviews were conducted by two psychiatrists and one psychologist trained in the use of the K-SADS. Both parents and children were interviewed.
Neuropsychological measures

A one hour neuropsychological battery was administered to each subject by a trained psychologist and comprised the following:

(1) The word reading subtest of the wide range achievement test 4 (WRAT-4) was used as a proxy for general intelligence.6

(2) Motor skills were tested using a modified version of the Purdue Pegboard task7 which required individuals to place metal pegs into slots on a wooden board as quickly as possible. The number of slots filled in 30 seconds was recorded and the participants completed three trials per hand which were averaged for the final measure. For analysis, the average score of the dominant and non-dominant hands was used. Hand dominance was assessed by Edinburgh Handedness Inventory.8

(3) Verbal working memory was assessed using the backward span of the Wechsler Digit Span Task.9 Short-Term Memory was assessed using the forward span. Digits were verbally presented to the participants at a rate of one per second.

(4) The Controlled Oral Word Association Test is a subtest of the verbal fluency task that required participants to name as many words possible starting with the letters F, A, and S within one minute per letter.10

(5) The British Picture Vocabulary Scale was used to test receptive language. A series of 4 images were shown simultaneously on a sheet of paper and the participants were asked to name the image which corresponded to a word that was verbally presented by the experimenter.11

(6) The Similarities subtest of the Wechsler Intelligence Scale for Children was used to assess abstract reasoning.9

(7) The Sustained Attention to Response Reaction Time task is 'go no-go' paradigm that tests attention.12 The digits from '1' to '9' were presented in a cyclic repeating
pattern on screen and participants were asked to make a button press response to all digits except ‘3’. Errors of commission were used for the analysis.

(8) The Trail-Making Test A and B assesses visuomotor abilities, speed of processing and cognitive flexibility. In Trails-A subjects are asked to connect a series of numbers and in Trails-B subjects are asked to connect a series of letters and numbers consecutively.

**Statistical analysis**

The demographic variables of age and sex were compared between the groups using *t*-test and chi-square test respectively. The analysis was carried out in two steps. In the first step, means of the raw scores were compared between the symptomatic and comparison groups using independent *t*-tests. Subsequently *Z*-scores for all of the neuropsychological scores were computed using the mean and standard deviation (SD) of the control group: \( \frac{X - \text{mean of control group}}{\text{SD of control group}} \). A multivariate analysis on the standardised variables yielded a predictive neurocognitive model adjusted for age (in months) and gender. SPSS-15 was used for the analyses.

**Results**

The symptomatic group differed from the comparison group on age (144 months (SD=6.9) vs 151 months (S.D.=4.2); \( t(35) = 3.5, p<0.001 \)). The gender difference did not reach statistical significance but there was a preponderance of males in the symptomatic group (41% vs 25% in the comparison group). Table 1 shows the results of the univariate and multivariate analyses. In the univariate analysis, adolescents who reported subclinical psychotic symptoms performed significantly worse than the comparison group on the receptive language test, and on the task assessing motor skills. In the multivariate analysis adjusted for age and
gender, the symptomatic group differed from the comparison group on the receptive language task, the motor task and the Trail-Making Test part B.

Table 1
Neuropsychological scores in adolescents at-risk with subclinical psychotic symptoms (n=17) and comparison, healthy control adolescents (n=20): results of univariate analyses and multivariate analysis adjusted for age and gender.

<table>
<thead>
<tr>
<th>Test Variable</th>
<th>Raw scores Controls</th>
<th>Raw scores At-risk</th>
<th>Univariate analysis</th>
<th>Multivariate analysis (adjusted for age and gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>t</td>
<td>df</td>
</tr>
<tr>
<td>WRAT read</td>
<td>51.3 (5.4)</td>
<td>51.6 (4.5)</td>
<td>0.17</td>
<td>35</td>
</tr>
<tr>
<td>BPVS</td>
<td>124.8 (12.3)</td>
<td>114.5 (5.4)</td>
<td>-3.1</td>
<td>32</td>
</tr>
<tr>
<td>Pegboard</td>
<td>13.7 (1.2)</td>
<td>12.9 (1.3)</td>
<td>-1.9</td>
<td>34</td>
</tr>
<tr>
<td>Similarities</td>
<td>26.15 (5.34)</td>
<td>24.1 (3.6)</td>
<td>-1.4</td>
<td>35</td>
</tr>
<tr>
<td>Digit forward</td>
<td>11.1 (1.8)</td>
<td>10.6 (2.2)</td>
<td>-0.73</td>
<td>34</td>
</tr>
<tr>
<td>Digit backwards</td>
<td>8.4 (1.7)</td>
<td>8.9 (2.1)</td>
<td>0.67</td>
<td>34</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>24.2 (6.2)</td>
<td>24.9 (7.4)</td>
<td>0.31</td>
<td>32</td>
</tr>
<tr>
<td>Attention</td>
<td>3.5 (1.6)</td>
<td>4.2 (1.8)</td>
<td>1.15</td>
<td>32</td>
</tr>
<tr>
<td>Trails A</td>
<td>36.4 (1.6)</td>
<td>41.7 (14.8)</td>
<td>0.86</td>
<td>26</td>
</tr>
<tr>
<td>Trails B</td>
<td>48.9 (13.6)</td>
<td>60.6 (24.5)</td>
<td>1.5</td>
<td>26</td>
</tr>
</tbody>
</table>

Discussion

We report that adolescents with sub-clinical psychotic symptoms display subtle but significant deficits in receptive language, motor skills and executive function. Similar deficits have been shown to precede adult onset schizophrenia-spectrum disorders in birth cohort studies.\textsuperscript{15,16,17} Receptive language deficits have also been linked with later schizophrenia-spectrum disorders in a follow-up study of
children with language disorders.\textsuperscript{18} Motor dysfunction, in particular, is one of the most consistently-replicated developmental precursors of schizophrenia and has also been reported in children at genetic risk for schizophrenia and in studies of archived videotaped footage.\textsuperscript{19}

A recent study from the ALSPAC cohort reports a non-linear association between IQ and psychosis-like symptoms in the ALSPAC birth cohort such that symptoms were associated with low IQ only.\textsuperscript{3} We did not find a relationship between IQ and subclinical psychotic symptoms in this sample but all of our sample were within the normal range for IQ and attending normal schools.

Our findings on the motor task and the Trail-Making task overlap substantially with emerging results on neurocognitive impairment in prodromal samples.\textsuperscript{20,21} Taken together these findings suggest continuity in the pattern of neurocognitive compromise seen in adolescents with subclinical symptoms and those in the prodrome for psychosis. Receptive language, fine motor skills and cognitive set shifting require co-ordinated activity of fronto-temporal regions and reports show that these regions are compromised in individuals who later make the transition to psychosis.\textsuperscript{22} Our study highlights the need for longitudinal follow-up to determine which neurocognitive deficits are most predictive of transition to psychosis. These findings, if replicated, provide clues to identifying the underlying brain structural and functional deficits that may identify those at increased risk for psychosis. Further elucidation of the brain regions or networks that subserve these functions will help elucidate the neural trajectory to psychosis and inform the search for preventive interventions.
References


Appendix Six

ABD study: Children’s consent form

Dear _

You might remember that you took part in our study on Adolescent Brain Development carried out at your school on_.

As we explained at the time, we are going on to do further research on this topic and you indicated that you wished to be considered for participation in this project.

I have included information here about what these parts of the study will involve for you and your parents/guardians to read through.

I will telephone your parents/guardians over the next few days to discuss these sections of the research and to answer any questions.

Kind regards
What will happen if I take part?
The part of the study will involve going to the famous Trinity College Dublin where we can take photographs of your brain. We will show you around our laboratory and you will get to see the state-of-the-art scientific equipment being used in the university. We will ask you to do some more tasks like the ones you carried out during the first part of the study, as well as answering some questions. We will then take some photographs of your brain, using an MRI scanner. This is a new and very sophisticated machine that, using magnets, can make accurate images of your brain. The scanner is shaped like a big white donut, with a hole in the middle where people can place their heads. You will lie down on a bed and the top part of your body will go inside this hole so that your head is in the middle. We will give you a set of earphones so that you can hear us clearly while you are inside (the machine is quite noisy!). Inside this hole there will also be a TV screen where we will show you some words and pictures. Then we will ask you to push some buttons in response to them. Each brain picture takes between 5 and 10 minutes to measure and you will have to stay as still as possible so that we get a clear picture (moving about causes the pictures to be blurry, just like moving during a regular photograph). We will explain everything fully before we start, and your Mum or Dad or guardian will be able to come with you. You will even get to practice in a fake machine that looks just like the real one. The scan will take about 45 minutes in total. (If you would like to learn more about MRI scanners, you can check the following web page on the internet: http://psych.wisc.edu/childemotion/mri.html)

In addition to the MRI scan you will also the opportunity to have an Electroencephalogram (EEG). This equipment is located in the same building as where you will have the MRI scan at Trinity College. To record EEG what we will do is place a soft cap over your head (similar to a swimming cap, but not as tight) and there are several holes in the cap for the electrodes (see picture). Electrodes measure the amount of electrical voltage of the cells in your brain. The electrodes snap into the holes in the cap and a special gel (similar to hair gel) is put in the holes before hand to ensure the activity from your scalp happens along the electrode. While we are putting on the cap, you can watch a DVD of your choice. When we have finished placing and preparing the cap, you will sit and watch a series of images on a computer monitor and make the responses with button presses. You can have your mum or dad with you throughout the study session if you like. If you start to get tired or bored, we will take short break or stop the study early. You may stop the study for any reason at any time. When the session is over, we will remove the cap and gently cleanse your hair or if you would rather wash it yourself the gels washes out easily with normal shampoo. The entire EEG session takes about one hour, but the actual amount of time you will be watching the computer while wearing the cap is around 30 minutes.
This EEG cap allows us to measure brain activity so that we can study how the brain works while it is processing information. Although EEGs used for recording human brain activity has been used for several decades for both medical and research purposes, we still know very little about adolescent brain development and neural responses to different types of stimuli. The information collected from you during this session will have important implications for our understanding of how adolescent’s brains process the things they see.

Is there anyone who cannot take part in this section?
Yes. If you are female and you think you might be pregnant, you unfortunately cannot take part in this section. To be part of the study you cannot have been involved in any activity that might cause you to become pregnant. It is also very important that you tell us if you have any metal objects on you or in your body. This is because the MRI machine acts like a huge magnet. We have given your parents a list of the types of things that we must check you do not have when you are entering the scanner.

Will it hurt?
No! There is nothing that will cause you any pain or discomfort. We hope you will find it fun and exciting! But if you do not like any part of the study, just say so. You can stop at any time.

If you would like to take part, we have a form for you to sign to say so.

If you have any questions, your parents or guardians can ring the doctor in charge: Professor Mary Cannon, Consultant Psychiatrist, Beaumont Hospital; Associate Professor, Royal College of Surgeon’s in Ireland. Telephone: 01-809-3855 or alternatively: Sarah Jacobson; PhD Research Student; Trinity College Dublin, Trinity College Institute of Neuroscience, Llyod Building, Dublin 2. Telephone: 01-896-8417. Email: jacobsos@tcd.ie.
Consent form for Under 16s
Adolescent Brain Development Study

• I have read and understood the Information Leaflet
• I would like to take part in this study about Adolescent Brain Development

Your full name (Block capitals): ..............................................
Your signature: ......................................................
Today’s date: ......................................................
Appendix Seven

ABD study: Parent/Guardian consent form

Dear Parent/Guardian

You might remember that your son _ took part in our study on Adolescent Brain Development carried out at his school on _.

As we explained at the time, we are going on to do further research on this topic and _ indicated that he wished to be considered for participation in this project.

I have included information here about these parts of the study for you to read through. There is also an information sheet for _ to read through.

I will telephone you over the next few days to discuss these sections of the research with you and to answer any questions you may have. In the mean time, please feel free to contact me with any questions.

Kind regards
What will happen in this part of the study?
The second part of the study will involve bringing you to Trinity College Dublin where we can take photographs of your child’s brain. We will show you around our laboratory there and you will get to see the state-of-the-art scientific equipment being used in the university. In order to get more detailed and useful information, we will ask you to do some more tasks like the ones you carried out during the first part of the study, as well as asking some questions. We will then take some photographs of your brain, using an MRI scanner. You may have seen in the news that Trinity College recently acquired a state-of-the-art machine called an MRI scanner that can take pictures of people’s brains. One of the great things about MRI scanners is that, unlike some other imaging machines, they give off no radiation and have no associated long-term health risks. The MRI scanner is shaped like a big white donut, with a hole in the middle where people can place their heads. Your child will lie down on a bed and the top part of his body will go inside this hole so that his head is in the middle. We will give him a set of earphones so that he can hear us clearly while inside the scanner (the machine is quite noisy!). Inside this hole there will also be a TV screen where we will show him some words and pictures. Then we will ask him to push some buttons in response to them. Each brain picture takes between 5 and 10 minutes. (If you would like to learn more about MRI scanners, you can check the following web page on the internet: http://psych.wisc.edu/childemotion/mri.html).

In addition to the MRI scan your child we also take part in an Electroencephalogram (EEG). This equipment is located in the same building as the MRI scanner at Trinity College. To record EEG we will place a soft cap over your child’s head (similar to a swimming cap, but not as tight) and there are several holes in the cap for the electrodes (see picture). This cap allows us to make noninvasive recordings of brain. The electrodes snap into these holes and a special electrolyte gel (similar to hair gel) is put in the holes before hand to ensure the signal picked up on the scalp is conducted along the electrode. While we are putting on the cap, your child will be entertained by a DVD of their choice. When we have finished placing and preparing the cap, your child will sit and watch a series of images on a computer monitor and make the appropriate responses with button presses. You can be with your child throughout the study session if you like. If your child becomes tired or restless, we will take short breaks to cheer him or her up or stop the study early. You may stop the study for any reason at any time. When the session is over, we will remove the cap and gently cleanse your child’s scalp and hair or if your child wishes to do it them self washing the hair with normal shampoo easily washes out the gel. The entire EEG session takes about one hour, but the actual amount of time your child will be watching the computer while wearing the cap is around 30 minutes.
The cap allows us to measure brainwaves so that we can study how the brain works while it is processing information. Although these procedures for recording human brain activity have been used for several decades for both medical and research purposes, we still know very little about adolescent brain development and neural responses to different types of stimuli. The information collected from your child in this will have important implications for our understanding of how adolescent’s brains process visual information.

**Will it hurt?**
No! There is nothing in the study that will cause any pain. We hope he will find it fun and exciting! But if he does not like any part of the study, he can just say so and we will stop at any time.

**Will there be any costs involved?**
No, we will reimburse you any expenses involved in taking part in this study and you will be recompensed for your time.

**Our responsibilities to you as investigators**
If the investigators become aware of any information during the course of the study that may affect your willingness to continue to participate you will be told immediately.
Further information on MRI Scanning:

What to expect in the MRI scanner

During MRI scanning, your child will be in contact with the MRI operator via an auditory communication system (essentially, a walkie talkie). This will be used to check that they are comfortable and to allow them to communicate back with us. There will also be a button in the scanner that they can press at any time to indicate that they wish to stop the scanning procedure.

The noise produced by an MRI scanner can be very loud so we will give your child headphones or earplugs to protect their hearing. A small percentage of people have reported that they were not as good at hearing quiet sounds for a short period after being in the scanner (this is called a temporary threshold shift). In the small number of cases where this has occurred it has resolved by itself after a short period of time.

The tube of the MRI scanner (where the top half of your child’s body will be placed) is a bit tight for space. People who do not like small spaces may feel a little claustrophobic while in there. If your child has experienced claustrophobia (fear of tight spaces) in the past, please let us know.

For female participants it is also important that they tell us if there is any possibility that they may be pregnant. To date there are no known risks of MRI during pregnancy, however as a precautionary safety measure pregnant individuals will not be included in the study. To participate in the current study participants of childbearing potential must be abstinent from sexual activity.

For your child’s comfort, we suggest that they wear loose-fitting, comfortable clothing, preferably with no metal zippers around the neck or near the head. They should bring only the minimum number of jewellery items (watches, rings, earrings, etc.), as all of these must be removed before scanning.

Further information on EEG:

What is EEG?

To give you a background on this noninvasive technique I will describe below what is taking place during the EEG. When brain cells fire, they release tiny amounts of electricity that travel right through the brain and the scalp. This is very convenient because it means that we can measure a person’s brain responses from outside their head. We do this by using small sensors that are placed near the scalp. Unfortunately, these sensors do not just pick up electricity from brain cells. They also detect random electricity produced by other types of cells such as muscle cells around the eyes and jaw. To get rid of this unwanted random electricity, we take a continuous recording (called an electroencephalogram or EEG) of the electricity present at a person’s scalp (i.e., brain-cell electricity + random electricity) while we present them with the same stimulus over and over again. We then average together time chunks of the EEG that occur when the stimuli occur. When any type of random activity is averaged together, it cancels itself out. So, when we average the EEG chunks, the random electricity cancels itself out, leaving us with the brain-cell electrical potentials that are related to a particular stimulus event. This method of analysis is referred to as ERP. It is important to note that when electricity travels through the head it gets deflected by the different layers of brain tissue and bone. This makes it difficult to use ERPs to find the location of firing brain cells. However, the deflections do not interfere with the
timing of the electrical potentials. So ERPs are good for measuring the temporal processing of perceptual stimuli (e.g., sounds, images, tastes), motor responses (e.g. a button press), and higher level cognitive skills.

**Are there any risks or potential risks associated with the study?**

When operated by appropriately qualified individuals, MRI and EEG present virtually no risk. There is **NO** exposure to x-rays or radioactivity with this procedure.

The MRI scanner uses a very strong magnetic field to take pictures. Because of this magnetic field, it would be dangerous to take any metal into the scanner (as it would lunge towards the centre of the machine, possibly hitting someone). For this reason, **it is very important that your child tells us if they have any metal on or inside their body.** We will go through a full safety screening procedure with you before you go into the MRI scanner where we will check that you have no metal but for your information, we have included a list of some of the metal items that should be removed before going into the scanner towards the end of this information form.

Please note that people with any of the following should **not** undergo an MRI procedure:

- Cardiac pacemaker or an implanted defibrillator
- Catheter that has metal components that may pose a risk of a burn injury
- A metal clip placed to prevent bleeding from an intra-cranial aneurysm
- A medication pump (such as that used to deliver insulin or a pain-relieving drug)
- A cochlear (inner ear) implant

It is essential that you inform us if your child has any metal items.

In very rare cases, the strong magnetic field can stimulate nerve cells. This may, for example, cause a muscle to twitch, while the magnetic field is turned on (this will stop once the magnetic field is turned off). In other very rare cases, it has been reported that the radio signals caused burns. As with all technology, there is always a possibility that unknown risks will become associated with MRI. However, MRI has now been used extensively for over 10 years with children and adults and there are no long-term health risks associated with it.

**What if the brain imaging finds some abnormality in my child's brain?**

Serious brain abnormalities are rare among people without a medical history of such problems. The brain images that we will take are for research purposes and are not the kind that would necessarily be used for clinical diagnosis. We will not routinely check images for the presence of a brain abnormality. However, should an abnormality be detected, we will contact you immediately and will recommend that you contact your GP to arrange for a clinical-quality brain scan. To ensure that you can be contacted at a later date, you will be asked to provide a name and contact details for a next-of-kin.

Although a significant abnormality is extremely unlikely, you should be aware that if such an abnormality is detected and you are informed, then this knowledge might have consequences for your child. Please take the time to consider carefully what it would mean to you if we told you of an abnormality in your child’s brain which
might, or might not, affect them later in life. Knowledge of an abnormality may affect their ability to work in certain professions, obtain life or health insurance and other facets of daily living. On the other hand, this information would allow your child to access medical treatment that would not otherwise have been considered and which may be beneficial for such a condition. If you would rather not know about a brain abnormality, then it is better not to participate in this study.

Your GP will be informed of your participation in this study. If any medically important information about your child’s brain function is learned in the course of this study you and your child’s GP will be informed.

All information collected during this study will be kept in the strictest confidence. Participants will be assigned a code number following their first contact with the researcher. This number will be used throughout the experiment and will be the only identifier on behavioural and physiological data and magnetic resonance (MR) scans; the identity of participants will not be revealed at scientific meetings, in publications or anywhere else.

For your information, your participation in this study will be fully covered by an approved policy of insurance. The insurance brokers for Trinity College Dublin are Coyle Hamilton Willis and professional indemnity for all research activity is with Royal Sun Alliance.

IF YOU REQUIRE FURTHER INFORMATION
If you have any further questions about the study, or if you wish to withdraw from the study you may do so without justifying your decision. For additional information now or any future time please contact:
Names: Professor Mary Cannon
Consultant Psychiatrist,
Beaumont Hospital,
Associate Professor
Royal College of Surgeons in Ireland
Phone No: 01 809 3855

Sarah Jacobson
PhD Research Student
Trinity College Dublin
Trinity College Institute of Neuroscience, Llyod Building, Dublin 2
Phone No: 01 896 8417
Email: jacobsos@tcd.ie
Some items that should not be brought into scanner

Items that **must be** removed by individuals before entering the MRI facility include:

- Purse, wallet, money clip, credit cards, cards with magnetic strips;
- Electronic devices such as beepers or cell phones;
- Hearing aids;
- Metal jewellery (in all parts of the body), watches;
- Pens, paper clips, keys, coins;
- Hair barrettes, hairpins;
- Any article of clothing that has a metal zipper, buttons, snaps, hooks, underwire bras, or metal threads;
- Shoes, belt buckles, safety pins.

Other objects that may be hazardous include:

- Metallic spinal rod
- Plates, pins, screws, or metal mesh used to repair a bone or joint
- Joint replacement or prosthesis
- Metal jewelry such as that used with body piercing.
- Some tattoos or tattooed eyeliner (these alter MR images, and there is a chance of skin irritation or swelling; black and blue pigments are the most troublesome)
- Bullet, shrapnel, or other type of metal fragment
- Metallic foreign body within or near the eye (such an object generally can be seen on an x-ray; metal workers are most likely to have this problem)
- Dental fillings (while usually unaffected by the magnetic field, they may distort images of the facial area or brain; the same is true for orthodontic braces and retainers)

**If your child has any of these items, please inform us before entering the scanner.**
Parent/Guardian Consent Form

I confirm that:

- I have been informed of the discomforts and risks that my child may reasonably expect to experience as part of this study.
- I have been informed that if a brain abnormality is observed, that I will be contacted for a meeting with a doctor.
- I have been informed that when used on appropriately qualified individuals, MRI presents virtually no risk. There will be no exposure to x-rays or radioactivity in this study.
- I understand that noise produced by this exam could be very loud, and that my child will wear earplugs or headphones to prevent damage to their hearing. Even with earplugs, the noise produced by the exam may produce temporary threshold shifts (i.e., temporary decreased ability to hear quiet sounds).
- I have been informed that my child may experience some discomfort from lying in the MRI scanner such as claustrophobia (fear of being closed in a tight space) or tight sensations from having their head restrained to minimise movement.
- I have been informed that my child will be asked to perform some tasks that they have been trained on, prior to the MRI procedure, which should not cause undue distress.
- I have been informed that other risks of injury due to MRI include damage to implanted electronic devices (such as pacemakers), haemorrhage if aneurysm clips are present and trauma if metal objects are brought too close to the scanner. However, these risks are minimal in a properly administered site. My child does not have any of these items in his/her body.

- I consent to my child’s participation in this research.
- I understand that I can withdraw my child at any time from the study.

Name (Block Capitals).................................................................

Signature .................................................................

Today’s date .................................................................
Appendix Eight

ChSz study: Letter of information to patients

Thank you most sincerely for your interest in our research. You may have already participated in research examining various aspects of memory and problem solving. Your time and participation is most appreciated.

This research seeks to investigate the genetics of mental health. One aspect of this research is to determine whether genes thought to be involved in mental health have a specific role in brain function e.g. memory and attention. More specially, we are looking at the way in which the structure and function of the brain is influenced by genetics.

The current study uses a test called an MRI. MRI, or magnetic resonance imaging, it is a non-invasive method of obtaining images of internal soft bodily tissue such as the brain and the spinal cord through the use of powerful magnets and radio waves.

The preliminary stages of your participation will involve preparing the equipment and ensuring that you remove all metal objects (e.g. belt, wallet etc.). Thereafter, the actual time spent in the scanner will be under 60 minutes. During this time scan of your brain will be taken. A brain scan will also be conducted while you complete two memory tasks, each of which will take approximately 10 minutes and one task of passive emotion, which involves no response, which will take approximately 10 minutes.

Now that you have read this information, please take the opportunity to ask the experimenter any questions you have about the general nature of the study and your participation in it.

Sincerely,

Sarah Jacobson
Ph.D Research Student
Trinity College Dublin
Trinity College Institute of Neuroscience, Office 3.01
Dublin 2
Phone: 087 960 1784
What are the risks associated with MRI?

When operated by appropriately qualified individuals, MRI presents virtually no risk, as there is NO exposure to x-rays or radioactivity with this procedure. The noise produced by an MRI exam can be very loud and you will be issued with protective headphones or earplugs to prevent damage to your hearing. The noise produced by the exam has been reported to produce temporary threshold shifts (i.e., decreased ability to hear quiet sounds) in a small percentage of people. Given the confines of an MRI machine, a small percentage of people in the past have reported feeling claustrophobic (fear of being closed in a tight space) when placed into an MRI scanner. Please let us know if you have experienced claustrophobia in the past.

During MRI scanning, you will be in contact with the MRI operator via an auditory communication system. This will be used to regularly check your comfort and to allow you to inform us of any problems or concerns. You will also have a "panic button" which you may press at any time to indicate that you wish to stop the scanning procedure.

As the MRI involves a large magnetic field, it is essential that NO METAL BE BROUGHT INTO THE SCANNER WITH YOU.

Items that must be removed by individuals before entering the MRI facility include:

- Purse, wallet, money clip, credit cards, cards with magnetic strips;
- Electronic devices such as beepers or cell phones;
- Hearing aids;
- Metal jewellery (in all parts of the body), watches;
- Pens, paper clips, keys, coins;
- Hair barrettes, hairpins;
- Any article of clothing that has a metal zipper, buttons, snaps, hooks, underwire bras, or metal threads;
- Shoes, belt buckles safety pins.

Other objects that may be hazardous include:

- Metallic spinal rod
- Plates, pins, screws, or metal mesh used to repair a bone or joint
- Joint replacement or prosthesis
- Metal jewellery such as that used with body piercing.
- Some tattoos or tattooed eyeliner (these alter MR images, and there is a chance of skin irritation or swelling; black and blue pigments are the most troublesome)
- Bullet, shrapnel, or other type of metal fragment
- Metallic foreign body within or near the eye (such an object generally can be seen on an x-ray; metal workers are most likely to have this problem)
- Dental fillings (while usually unaffected by the magnetic field, they may distort images of the facial area or brain; the same is true for orthodontic braces and retainers)

If you have any of these items, please inform us immediately.

Who shouldn’t undergo the MRI procedure?
Research participants who have the following items should not undergo an MRI procedure:

- Cardiac pacemaker or an implanted defibrillator
- Catheter that has metal components that may pose a risk of a burn injury
- A metal clip placed to prevent bleeding from an intra-cranial aneurysm
- A medication pump (such as that used to deliver insulin or a pain-relieving drug)
- A cochlear (inner ear) implant

It is essential that you inform the MR operator if you have any metal items in any of the above lists.

What if the brain imaging finds some abnormality in my brain?
The brain images that we acquire are obtained for research purposes and are not the kind that would necessarily be prescribed for clinical diagnosis. We will not routinely check images for the presence of a brain abnormality. However, should an abnormality be detected, we will contact you immediately and will recommend that you contact your GP to arrange for a clinical-quality brain scan. To ensure that you can be contacted at a later date, you will be asked to provide a name and contact details for a next-of-kin.

Although a significant abnormality is extremely unlikely, you should be aware that if such an abnormality is detected and you are informed, then this knowledge might have consequences for you. Knowledge of an abnormality may affect your ability to work in certain professions, obtain life or health insurance and other facets of daily living. If you do not want to know, then it is better not to participate in this study.
Appendix A

Structural and functional MRI protocol

Procedure: MRI-DTI acquisition: In vivo DTI will be acquired using a single-shot echo-planar imaging (EPI) sequence with SENSE parallel imaging scheme (SENSitivity Encoding). The diffusion weighting will be encoded along 15 independent directions, using a b value of 800 s/mm². The field of view (FOV) will be 230 x 230 mm, and TR/TE of 11061/47ms, resulting in an isotropic voxel size of 2 mm. Using 8 repetitions, the examination time will be about 6 minutes. Fractional anisotropy (FA) and mean diffusivity (as measured by the apparent diffusion coefficient, ADC) will be extracted with in-house software written in IDL (Interactive Data Language, Research Systems Inc., Colorado, USA) as the main dependent variables for this study. A T1-weighted 3D sequence with a FOV = 230 x 230 mm, 256 x 256 matrix, RT = 8.4ms, ET = 3.8ms, and slice thickness = 0.9 mm will be acquired in addition, with about 10 min. acquisition time.

Bold fMRI analysis
Whole brain BOLD fMRI data were collected on a Philips 3 Tesla Signa scanner acquiring 24 interleaved slices (echo time=30ms, repetition time=2 s, flip angle=90%, field of view=24 cm, matrix=64x64, voxel dimensions 3.75x3.75x6 mm) covering the whole brain. All fMRI data were processed and spatially normalized to a common stereotaxic space (Montreal Neurologic Institute template) and analyzed using a random effects model within SPM99 software (Brett, 2004; Wellcome Department of Cognitive Neurology, 2000).

Task to complete during the MRI
There will be a practice session before the scan in which the instructor will show the subject how to successfully complete the task. Subjects will then do the same task while in the scanner. They will see the task presented on a screen and the instructor will show them how to respond. The practice should take no more than 10 minutes to learn and will take less than 10 minutes to complete in the scanner.
Appendix Nine

ChSz study: Consent form

Title of Project:
"An fMRI investigation of error awareness in schizophrenia involving brain volumetries"

Information about the Project:
In this study we are seeking to gather information about the function, structure and metabolic compounds in specific regions of the brain. Our ability to monitor ongoing performance is critical to behavioural control in particular to the processing of errors. Looking at the brain whilst monitoring errors has become of interest in neuroscience as it offers insights into the dysfunctions of self monitoring seen in a range of clinical conditions. Executive functions are the skills involved in controlling behaviour. Examples of executive functions include the ability to stop a response that you have already started and to monitor your behaviour to detect how well you are doing on a task. In our study we will focus on error awareness. Error awareness is the ability to monitors ones own performance. To identify the brain regions involved in awareness of ones error, you will be asked to perform some tasks while we image your brain using Magnetic Resonance Imaging (described below). Prior to the imaging, you will be asked to practice the tasks so you will know what to do once in the scanner.

Task Description
You will be asked to complete a task that measures error awareness and inhibitory control, reaction time and accuracy. You will be given the information on how to complete the tasks then asked to do a practice on a computer to show that you understand the tasks. You will then do the same tasks while in the scanner. You will see the tasks presented on a screen and the instructor will show you how to respond. These tasks should take no more than 30 minutes to learn and will take around 10 minutes to complete in the scanner.

The study will take place at the MRI facility located at Trinity College Dublin. The session (including the MRI scanning) will take 2.0 hours. If at any time during the research you wish to withdraw from the study then you may. All information gathered during the course of this research is available to you upon request, and will be held in the strictest of confidence. Please ask the researcher any questions you may have. If you are happy to be involved in this study, then please complete this consent form and make it available to the researcher(s).

If, at any time, you have any questions regarding this research, please feel free to contact Sarah on 087 960 1784.
What is MRI?

The purpose of functional MRI scanning is to determine which brain regions are activated as someone performs certain tasks. In the MRI scanner there is a very large magnetic field. This magnetic field and radio signals which are transmitted in the scanner measure the concentration of water particles within the body, allowing brain functioning relating to behaviour to be measured in terms of blood flow to the brain. The person who is going to be scanned lies on a bed where their head is placed into a device which has the appearance of a large helmet. When the person has been safely and comfortably secured in this device, the bed is moved slowly into the scanner. When the person’s head is in the middle of the magnetic field, radio frequency pulses and magnetic fields are switched on and off to produce a signal which we use for measuring blood flow.

Individual MRI test runs will last no longer than 10 minutes to minimise fatigue and the entire testing session will be completed within 60 minutes. It is very important that you keep still and, in particular, do not move your head while we are taking an image of your brain. For some images, you will be doing a cognitive task that you will have practiced outside of the scanner. For other images, you will just lie still and relax while we take high-resolution images of your brain. We will explain exactly what you need to do before we start each MRI test run.

What will I be asked to do while I am in the MRI scanner?

You will be asked to perform cognitive tasks that you will have already practiced with one of the researchers prior to the scanning session. During scanning, we will tell you what task to do before each scan by communicating through the intercom system.

What are the risks associated with MRI?

When operated by appropriately qualified individuals, MRI presents virtually no risk, as there is NO exposure to x-rays or radioactivity with this procedure. The noise produced by an MRI exam can be very loud and you will be issued with protective headphones or earplugs to prevent damage to your hearing. The noise produced by the exam has been reported to produce temporary threshold shifts (i.e., decreased ability to hear quiet sounds) in a small percentage of people. Given the confines of an MRI machine, a small percentage of people in the past have reported feeling claustrophobic (fear of being closed in a tight space) when placed into an MRI scanner. Please let us know if you have experienced claustrophobia in the past. During MRI scanning, you will be in contact with the MRI operator via an auditory communication system. This will be used to regularly check your comfort and to allow you to inform us of any problems or concerns. You will also have a "panic button" which you may press at any time to indicate that you wish to stop the scanning procedure.

As the MRI involves a large magnetic field, it is essential that NO METAL BE BROUGHT INTO THE SCANNER WITH YOU.

Items that must be removed by individuals before entering the MRI facility include:
Purse, wallet, money clip, credit cards, cards with magnetic strips;
Electronic devices such as beepers or cell phones;
Hearing aids;
Metal jewellery (in all parts of the body), watches;
Pens, paper clips, keys, coins;
Hair barrettes, hairpins;
Any article of clothing that has a metal zipper, buttons, snaps, hooks, under wire bras, or metal threads;
Shoes, belt buckles safety pins.

Other objects that may be hazardous include:

- Metallic spinal rod
- Plates, pins, screws, or metal mesh used to repair a bone or joint
- Joint replacement or prosthesis
- Metal jewellery such as that used with body piercing.
- Some tattoos or tattooed eyeliner (these alter MR images, and there is a chance of skin irritation or swelling; black and blue pigments are the most troublesome)
- Bullet, shrapnel, or other type of metal fragment
- Metallic foreign body within or near the eye (such an object generally can be seen on an x-ray; metal workers are most likely to have this problem)
- Dental fillings (while usually unaffected by the magnetic field, they may distort images of the facial area or brain; the same is true for orthodontic braces and retainers)

If you have any of these items, please inform us immediately.

There may be additional or unknown risks associated with MRI. For example, in very rare cases, the strong magnetic field can induce nerve stimulation (e.g., switching the strong magnetic field gradients during imaging has been reported to cause twitching in the neck muscles). Also, in very rare cases, the radio signals have been reported to cause burns. There may be other risks associated with imaging that are not yet known.

Who shouldn’t undergo the MRI procedure?

Research participants who have the following items should not undergo an MRI procedure:

- Cardiac pacemaker or an implanted defibrillator
- Catheter that has metal components that may pose a risk of a burn injury
- A metal clip placed to prevent bleeding from an intra-cranial aneurysm
- A medication pump (such as that used to deliver insulin or a pain-relieving drug)
- A cochlear (inner ear) implant
It is essential that you inform the MR operator if you have any metal items in any of the above lists.

**Pregnancy and MRI**

For female participants it is also important that you tell us if there is any possibility that you are pregnant. To date there are no known risks of MRI during pregnancy, however as a precautionary safety measure pregnant individuals will not be included in the study. To participate in the current study women of child-bearing potential must be using one of the following acceptable methods of birth-control:

a. oral or transdermal contraceptives  
b. barrier (diaphragm or condom) with spermicide  
c. intrauterine progesterone contraceptive system  
d. Levonorgestrel implant  
e. Medroxyprogesterone acetate contraceptive injection  
f. complete abstinence from sexual activity

**What if the brain imaging finds some abnormality in my brain?**

The brain images that we acquire are obtained for research purposes and are not the kind that would necessarily be prescribed for clinical diagnosis. We will not routinely check images for the presence of a brain abnormality. However, should an abnormality be detected, we will contact you immediately and will recommend that you contact your GP to arrange for a clinical-quality brain scan. To ensure that you can be contacted at a later date, you will be asked to provide a name and contact details for a next-of-kin.

Although a significant abnormality is extremely unlikely, you should be aware that if such an abnormality is detected and you are informed, then this knowledge might have consequences for you. Please take the time to consider carefully what it would mean to you if we told you of an abnormality in your brain which might, or might not, affect you later in life. Knowledge of an abnormality may affect your ability to work in certain professions, obtain life or health insurance and other facets of daily living. If you do not want to know, then it is better not to participate in this study.

**By providing my consent I agree that:**

I have been informed of the discomforts and risks that I may reasonably expect to experience as part of this study. I have been informed that if a brain abnormality is observed, that I will be contacted for a meeting with a radiologist. I have been informed that when used on appropriately qualified individuals, MRI presents virtually no risk. There will be no exposure to x-rays or radioactivity in this study. I understand that noise produced by this exam could be very loud, and that I will wear earplugs or headphones to prevent damage to my hearing. Even with earplugs, the noise produced by the exam may produce temporary threshold shifts (i.e., decreased ability to hear quiet sounds). I have been informed that I may experience some discomfort from lying in the MRI scanner such as claustrophobia (fear of being closed in a tight space) or tight sensations from having my head restrained to prevent
movement. I have been informed that I will also be asked to perform some tasks that I have been trained on, prior to the MRI procedure, which should not cause undue distress.

I have been informed that other risks of injury due to MRI include damage to implanted electronic devices (such as pacemakers), haemorrhage if aneurysm clips are present and trauma if ferrous metal objects are brought too close to the scanner. However, these risks are minimal in a properly administered site. **I do not have any of these items in my body.**

I understand these risks and am agreeing to volunteer to participate in this research. I understand that I can withdraw at any time from the study.

PARTICIPANT'S NAME: ________________________________

PARTICIPANT'S SIGNATURE: ___________________________

Date: __________________________

WITNESS'S NAME: ________________________________

WITNESS'S SIGNATURE: ___________________________

Date: __________________________

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Appendix Ten

IMAGEN-Psy study: Preliminary report

Introduction

The IMAGEN-Psy study is a sub-investigation of data collected in European Union commissioned IMAGEN study. The IMAGEN study is a European research project investigating mental health and risk-taking behavior in 2,000 14-year-old children, which involves research teams from England, France, Ireland, and Germany. I am currently leading an analysis of data collected from the London and Dublin recruitment sites, which have both added the very brief APSS questionnaire to their neuropsychological batteries to identify adolescents who are at heightened risk of developing schizophrenia. I have defined the at-risk criteria for this study based on a sensitivity and specificity analysis of the APSS data collected in the ABD study. Each question from the APSS had a possible response of “no” (0 points), “maybe” (.5 points), or “yes, definitely” (1 point) (see Chapter Two, Figure 3.1). Figure 1 depicts the ROC curve results for the sensitivity and specificity of each of the seven APSS questions. An area under the curve analysis revealed that the highest indicator of sensitivity and specificity was the question: Have you ever heard voices that other people can't hear? This question revealed 88% probability ($p=.0001$) of predicting which subjects would be classified as at-risk, which was determined through the consensus ratings from the clinical interviews (K-SADS and SIPS). Of those identified as at-risk from the clinical interview 77% ($n=13/17$) answered “maybe” or “yes, definitely” to this screening question regarding hearing “voices.” All of the control participants (100%) who were interviewed ($n=20$) answered “no” to this question.
Figure 1. A sensitivity and specificity analysis of each of the seven questions from the Adolescent Psychotic-Like Symptom Screener (APSS). This analysis revealed that the question regarding auditory hallucinations: Have you ever heard voices or sounds no one else can hear? (purple line). This question was the most predictive of individuals who would also report a “definite” psychotic-like symptom during a follow-up clinical interview.

Additionally, all of the at-risk subjects who endorsed the APSS question regarding hearing voices also endorsed the question: Have you ever seen something or someone that other people could not see? This question alone had 75% probability ($p=.01$) of predicting those who would be identified as at-risk from the clinical interview. Therefore answering “maybe” or “yes, definitely” to both the auditory and visual hallucination questions seemed to be the most predictive screening questions of those who were actually experiencing subclinical psychotic symptoms, as assessed by the lengthy clinical interviews.
Participants

Currently, 226 adolescents from the London (n= 119) and Dublin (n= 107) IMAGEN sites have been screened with the APSS. I found that 22 participants (n= 14 London, n= 8 Dublin), which is 10% of the total sample, answered “maybe” or yes, definitely” to both of the APSS at-risk criteria questions. Of these participants 17 completed an MRI scan (n= 11 London, n= 6 Dublin). One at-risk participant from the London at-risk sample was excluded due to a low IQ score, which was assessed by the WISC-IV. Therefore, a total of 16 at-risk participants (n= 10 London, n= 6 Dublin) have been currently scanned and will be included in the MRI analyses. Matched controls have also been identified for these 16 at-risk subjects. Participants were matched for study location (i.e. Dublin or London), age, gender, WISC-IV total score, parental education, and parental ethnicity. All control subjects answered “no” for both of the APSS at-risk criteria questions. The demographics of the current sample are presented in Table 1. All participants were Caucasian with both parents of either Irish or English ethnicity.

The goal in collecting the neuroimaging data in the IMAGEN-PSY investigation is to determine what brain structure and brain function differences might exist between the at-risk and control phenotypes and to relate any observed differences to those observed in adults with chronic schizophrenia. In a separate study being conducted at Trinity College Dublin I have begun fMRI data collection on chronic schizophrenia patients (n= 7) and their controls (n= 10), which will continue until the same number of participants are collected as the IMAGEN-PSY investigation, than fMRI, VBM and DTI analyses will be conducted. All participants are completing a monetary incentive delay (MID) task. The MID task is meant to probe functional activity reward processing.
Table 1
IMAGEN-PSY demographics and APSS at-risk criteria scores.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>At-risk (n= 16)</th>
<th>Controls (n= 16)</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>14</td>
<td>14</td>
<td>n/a</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>69%</td>
<td>69%</td>
<td>n/a</td>
</tr>
<tr>
<td>Gender (% female, n= 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average APSS total score for two at-risk criteria questions</td>
<td>1.34±.47</td>
<td>0 / (30)= -11.4</td>
<td>p=.000</td>
</tr>
<tr>
<td>Range: 1-2</td>
<td>Range: 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WISC-IV total score^a</td>
<td>164±28</td>
<td>164±26</td>
<td>t(30)=-.05.</td>
</tr>
<tr>
<td>Range: 117-201</td>
<td>Range: 122-196</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental education^b</td>
<td>4.4±2.4</td>
<td>4.1±1.9</td>
<td>t(30)=-.49</td>
</tr>
<tr>
<td>Range: 1-8</td>
<td>Range: 1-8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^aWISC-IV total score included total scores from the sub-tests: Block design, digit-span (longest forward and longest backward), matrix reasoning, similarities, and vocabulary.
^bParental education was valued by the relative degree achieved: 1=Professional qualification (i.e. Ph.D., M.D., M.Sc.), 2=Bachelor’s degree, 3=Advanced diploma, 4= A levels or BTEC, 5=NVQ or GNVQ, 6=O levels, GCSE, or CSE, 7=No school or primary school classes only, 8=None of the above.

FMRI task

In MID task, participants were shown one of three cues; each cue had a point value, and was shown during an anticipation phase before the onset of the target (a square) was presented. A cue which is a triangle informs the participant that they will be rewarded with zero points if they responded quickly enough to the target; a circle with one line through the middle is worth two points; and a circle with three lines in worth ten points. The location of the cue, on either the left or right side of the screen, cues the participant to which side the target was going to be presented on 100% of the time. An adaptive algorithm determined a 66% success rate of the participant’s performance.
Preliminary results

A preliminary analysis from 33 IMAGEN healthy control participants has revealed that during the anticipation phase when a participant was subsequently correct and awarded either two or ten points, there was increased activity in shows bilateral insula, bilateral ventral striatum, bilateral ventral tagmental area, bilateral midbrain nuclei, ACC and cerebellar vermis (Figure 2) (Personal correspondence Buehler, Thyreau, & Reed, 2009).

Figure 2. Reports preliminary analysis from the IMAGEN MID task (n= 33). Activations during the anticipation phase of the task are reported when a correct response was made after the presentation of a reward cue (either a two points or ten points cue). Significant increased activity was found in the bilateral insula, bilateral ventral striatum, bilateral ventral tagmental area, bilateral midbrain nuclei, ACC and cerebellar vermis. Reprinted data from a personal e-mail correspondence with IMAGEN colleagues Buehler, Thyreau, & Reed (2009).
Discussion

The comparisons of adult Sz patients with their adult-matched controls will serve as a positive control providing biomarkers of schizophrenia-related brain function and brain structure deficits. Results from this study will be able to probe activation in the regions of the ventral striatum and insula, which were not assessed in the response inhibition task in the ABD and ChSz studies, but revealed structural abnormalities. Additionally, VBM and DTI analyses will be carried out to determine the relationship between ventral striatum and insular functioning with results from these structural scans. It is hoped that the longitudinal assessment of the adolescents recruited in the IMAGEN project will provide follow-up information on the development of the disease enabling the ability to retrospectively predict who was likely to progress from being at-risk to developing psychosis. This knowledge will be valuable insofar as long-term outcomes are greatly improved with earlier identification/diagnosis. There is also concurrent collection of genetic material to allow for a subsequent genetic linkage analysis to allow for the identification of (endo)phenotypic measures associated with schizophrenia and/or a heightened risk of schizophrenia.