FUNCTIONAL BRAIN NETWORKS IN SCHIZOPHRENIA: MAPPING CONNECTIVITY AND TOPOLOGY AT EARLY AND LATE PSYCHOTIC ILLNESS STAGES

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Abstract

Schizophrenia is a severe mental disorder that is characterised by symptoms including hallucinations, delusions and disorganized thought. The cause of schizophrenia remains unknown; however, it is thought that a combination of genetics, environment and altered neurobiology play a role in the emergence and perpetuation of the disorder. Accumulating evidence suggests that disrupted brain network connectivity may in part underlie the pathophysiology of psychosis, and that network connectivity is to some extent genetically determined and heritable. However, there is still much to be learned surrounding the nature of network abnormalities and how they differ in early versus late psychosis. Exploring the underlying neurobiology at discrete clinical stages of psychotic illness creates a framework to evaluate the biological factors that may be contributing to the progression from early psychosis, to more advanced chronic stages of the disorder.

This thesis used resting-state functional magnetic resonance imaging (fMRI) to characterise network functional connectivity and topology in early and late psychosis, as well as in a group of unaffected family members (UFM) of individuals with schizophrenia. Resting-state fMRI is a well validated and sensitive tool for probing the intrinsic functional integrity of the brain. Specifically, this thesis used a data-driven approach to map the temporal coherence of fMRI time series (functional connectivity) across the whole brain. To complement the resting-state functional connectivity (rs-FC) analysis, this thesis used graph theory to explore functional network topology. Network topology describes that brains ability to maintain a balance between local processing speed and global integration of information. These methodological approaches were used to investigate network abnormalities in three groups

relative to healthy controls; a first-episode psychosis (FEP) group, a treatment-resistant schizophrenia (TRS) group and a group of UFM.

This thesis aimed to investigate 1) whether rs-FC and network topology was abnormal in the early FEP stage of schizophrenia relative to healthy controls at two time-points (baseline and at 12-months follow-up); 2) whether rs-FC and network topology was impaired in a chronic TRS group relative to healthy controls; 3) whether abnormal rs-FC and network topology was evident in a group of UFM, and whether any network measure could be characterised as a marker of risk or resilience to psychosis in UFM.

Firstly, results showed no evidence of abnormal rs-FC or topology in FEP individuals relative to healthy controls at baseline, or at the 12-months follow-up. Further, longitudinal changes in network properties over a 12-month period did not significantly differ between FEP individuals and healthy controls. Secondly, this thesis found widespread reductions in rs-FC in the TRS group that predominantly involved temporal, occipital and frontal brain regions. The TRS group also showed reduced global network efficiency and increased local efficiency relative to controls. Thirdly, TRS and UFM shared frontal and occipital rs-FC deficits, representing a 'risk' endophenotype. Additional reductions in frontal and temporal rs-FC appeared to be associated with risk that precipitates psychosis in vulnerable individuals, or may be due to other illness-related effects, such as medication. Functional brain networks were more topologically resilient in UFM compared to TRS, which may protect UFM from psychosis onset despite familial liability.

Together, the body of work presented in this thesis provides a number of novel and unique findings that serve to advance the current state of knowledge regarding the pathophysiology

and heritability of psychosis. Specifically, the work demonstrated that the latest most severe stage of psychosis, TRS, is associated with widespread reduced rs-FC, and that milder, yet similar patterns of dysconnectivity were observed in UFM, implying a genetic root to some, but not all of the observed network abnormalities. Network topology differed relative to healthy controls in both UFM and TRS patients, suggesting that functional network architecture is also disturbed in late psychosis, and again, results suggest a genetic/shared environmental basis for this characteristic. Our finding of no significant difference in rs-FC or network topology in our FEP sample suggests that there is a differentiation between biological processes occurring in early and late psychosis with a subgroup of individuals' rs-FC potentially being unaffected in the FEP stage.

Declaration

This is to certify that:

(i) the thesis comprises only my original work towards the PhD except where indicated,

(ii) due acknowledgement has been made in the text to all other material used,

(iii) the thesis is less than 100,000 words in length, exclusive of tables, figure legends, bibliography and appendices.

ELENI GANELLA

Signature: ……..

Date: 18-09-2017

Preface

The work in *Chapter 2* used data collected for a wider study, The Microglial Study. I would like to acknowledge and thank Vanessa Cropley for allowing me to use this resting-state data. I would also like to thank the meticulous work of Steven Tahtalian who was primarily involved in screening, assessing and scanning participants. Eleni Ganella was also involved in participant assessments, conducted all pre-processing and analyses of the data and interpretation of the results, and wrote the original as well as revised manuscripts in accordance with The University of Melbourne's doctoral thesis outlines. This work was published in the *Australian & New Zealand Journal of Psychiatry* on the 27th of May 2018.

The work in *Chapter 3* was published in the international journal *Schizophrenia Research* on the 20th of December 2016. Eleni Ganella was involved in assessing participants, conducted all pre-processing and neuroimaging and statistical analyses of the data and interpretation of the results, and wrote the original as well as revised manuscripts in accordance with reviewer and editorial comments. Author Andrew Zalesky designed the functional connectivity protocol and was imperative to the methodology and analysis of the neuroimaging data. Author Caio Seguin assisted in the design and execution of the graph theory analyses. Author Christos Pantelis, author Christina Phassouliotis and author Ian Everall were imperative to the design, recruitment and execution of the study. Author Sarah Whittle, author Chad Bousman and author Cali Bartholomeusz assisted in the statistical design of the study. All authors contributed to and have approved the final manuscript.

This work was presented at the Australasian Schizophrenia Conference in Melbourne, Australia, September, 2015, and the Schizophrenia International Research Society conference, in Florence, Italy, June 2016.

The data used for the work presented in *Chapter 4* was also part of the CRC for Mental Health research programme. This work is in press, and was accepted for publication in the journal *Schizophrenia Research* on the 6th of July 2017. The editor of *Schizophrenia Research* also wrote a letter commenting on the impact and importance of the paper. Eleni Ganella conducted all pre-processing and analyses of the data and interpretation of the results, and wrote the original as well as revised manuscripts in accordance with reviewer and editorial comments. Author Zalesky designed the functional connectivity, methodology and analysis of the neuroimaging data. Author Seguin assisted in the design and execution of the graph theory analyses. Author Pantelis, author Phassouliotis and author Everall were imperative to the design, recruitment and execution of the study. Author Whittle, author Bousman and author Bartholomeusz assisted in the statistical design of the study. Author Di Biase and author Wannan further assisted the statistical design of the study, recruitment of participants and management of functional resting-state data. This work was presented at the annual CRC for Mental Health Conference, April, 2017 and at the Melbourne Health Research Week, June, 2017.

Dedication

I would like to dedicate this thesis to all people living with schizophrenia, particularly those who selflessly participated in the studies that comprised this thesis. Without their courage and openness, there would be no thesis.

Acknowledgements

I would like to acknowledge the remarkable efforts of my amazing supervisors. Cali Bartholomeusz, we have been a team since my honours year in 2012 and you were a big factor in my decision to follow the research path. Thank you for your support over the past 6 years, I would never have reached this milestone without you. You have been more than a supervisor to me, you have been a mentor and a friend, and I value our time working together greatly. I would also like to thank Andrew Zalesky, the smartest person I have ever met. I never would have been able to perform such cool analyses and produce such great work without you. Thank you for always being so patient with me, and generous with your time and brain. I would also like to thank Christos Pantelis, who welcomed me into Melbourne Neuropsychiatry Centre 6 years ago, and has only encouraged and facilitated my growth and progression throughout all of my studies. It has been a joy working under such a supportive and intelligent professor. Sarah Whittle, thank you for always being there as a supportive supervisor, mentor and friend to help me in any way that I needed. Your wise and intelligent input and contribution to every chapter is so appreciated.

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Wannan. I cannot even begin to describe how much your friendship over the past 3.5 years has meant to me. You have been such an amazing support to me throughout this whole process. You not only kept me sane the whole time, but you made coming into work an absolute joy. I thank the Ph.D. Gods for blessing me with you as a Ph.D. sister every day, because I know that by going through this process together I have met in you a friend for life.

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List of Publications

First author articles resulting from this thesis:

Chapter 2:

Ganella, E. P., Seguin, C., Pantelis, C., Whittle, S., Baune, B., Olver, J., Amminger, P., McGorry, P. D., Cropley, V., Zalesky, A., Bartholomeusz, C. F., (2018). Resting-state functional brain networks in first-episode psychosis: a 12-month follow-up study. *Australian & New Zealand Journal of Psychiatry.* doi:10.1177/0004867418775833

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Co-authored articles applying methods and knowledge gained from this thesis:

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2. Chye, Y., Solowij, N., **Ganella, E. P**., Suo, C., Yucel, M., Batalla, A., Lorenzetti, V. (2017). Role of orbitofrontal sulcogyral pattern on lifetime cannabis use and depressive symptoms. *Progress in Neuropsychopharmacology andl Biological Psychiatry, 79*(Pt B), 392-400. doi:10.1016/j.pnpbp.2017.07.017

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Abbreviations

AAL: Automated Anatomical Labeling template

ACC: Anterior Cingulate cortex

ANCOVA: Analysis of Covariance

ANOVA: Analysis of Variance

AUC: Area Under the Curve

AVH: Auditory Verbal Hallucinations

BOLD: Blood-Oxygen-Level Dependent

BPRS: Brief Psychiatric Rating Scale

C: Controls

CAARMS: Comprehensive Assessment of At Risk Mental States

Cereb: Cerebellum

dlPFC: Dorsolateral Prefrontal Cortex

DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders 4th Edition

DUP: Duration of Untreated Psychosis

EOS: Early Onset Schizophrenia

FC: Functional Connectivity

FD: Framewise Displacement

FDR: False Discovery Rate

FEP: First-Episode of Psychosis

fMRI: Functional Magnetic Resonance Imaging

FOV: Field of View

Front = Frontal

FSL: FMRIB Software Library

Fus. G=Fusiform gyrus

FWE: Family-Wise Error

GAF: Global Assessment of Functioning

Gyr. Rec: Gyrus Rectus

Hesch. G: Heschl's Gyrus

IQ: Intelligence Quotient

Inf. Occ=Inferior occipital

Inf. Oper = Inferior operculum

Inf. Orb = Inferior orbital

Inf. Temp=Inferior temporal

Inf. Tri = Inferior triangularis

L: Left Hemisphere

MADRS: Montgomery Åsberg Depression Rating Scale

Med. Orb: Medial Orbital

mg: Milligrams

MHREC: Melbourne Health Human Research Ethics Committee

Midd. Front: Middle Frontal

Midd. Occ: Middle Occipital

Midd. Orb: Middle Orbital

Midd. Temp: Middle Temporal

Midd. T. P: Middle Temporal Pole

MINI: Mini International Neuropsychiatric Interview

MNI: Montreal Neurological Institute

mPFC: Medial Prefrontal Cortex

MPRAGE: Magnetization-Prepared Rapid Acquisition Gradient Echo

MRI: Magnetic Resonance Imaging (MRI)

NBS: Network-Based Statistic

Occ: Occipital;

Olf. Cor: Olfactory Cortex

OYH: Orygen Youth Health

PANSS: Positive and Negative Syndrome Scale

Par: Parietal

Para. Lob: Paracentral Lobule

PFC: Prefrontal Cortex

rs-FC: Resting-State Functional Connectivity

ROI: Region of Interest

RSN: Resting-State Network

rs-fMRI: Resting-State Functional Magnetic Resonance Imaging

R: Right hemisphere

Rol. Oper: Rolandic Operculum

SANS: Scale for the Assessment of Negative Symptoms

SCID: Structured Clinical Interview for DSM-IV-TR Axis I Disorders

SOFAS: Social and Occupational Functioning Assessment Scale

STG: Superior Temporal Gyrus

Sub = Subcortical;

Sup. Front: Superior Frontal

Sup. Med: Superior Medial

Supp. Mot: Supplementary Motor

Sup. Occ: Superior Occipital

Sup. Orb: Superior Orbital

Sup. Temp: Superior Temporal

TE: Time to Echo

Temp: Temporal

Temp. Pole: Temporal Pole

TR: Time to Repetition

TRS: Treatment-Resistant Schizophrenia

UHR: Ultra High Risk

UFM: Unaffected-Family Members

CHAPTER 1:

General introduction

1.1. **Schizophrenia: A brief introduction**

Although the term "schizophrenia" is less than 100 years old, the nonspecific concept of psychosis has existed for many thousands of years. Historical accounts of schizophrenia-like symptoms such as bizarre psychotic beliefs and behaviours were grouped under the blanket term 'madness', and date back to 1400 BC (Lewis, 1966). The disorder was first classified as a discrete mental illness in 1887 by Dr. Emile Kraeplin. Kraeplin grouped the symptoms that we now associate with schizophrenia, and called the disorder "dementia praecox", meaning a "premature dementia" associated with insanity (Wender, 1963). The term "schizophrenia" was later introduced by the Swiss psychiatrist Eugen Bleuler in 1911 (Bleuler & Bleuler, 1986), and replaced "dementia praecox" (given the disorder did not always lead to mental deterioration and could occur early as well as late in life) (D. W. Black & Boffeli, 1989). The word schizophrenia is of Greek origin, meaning split (schizo) mind (phrene), and was thought to describe the fragmented and disorganized thinking that is often associated with the disorder.

1.1.1. *Symptoms and diagnosis of schizophrenia*

Schizophrenia as we know it today, is a complex, severe, and debilitating mental illness that is hallmarked by disturbances in thought processes, emotion, and behaviour. Classified as a psychotic disorder within the Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5) (Battle, 2013), schizophrenia is characterised by six core symptoms; positive, negative, disorganization, cognitive, mood and motor symptoms (Battle, 2013). Positive symptoms describe added unnatural functions, such as hallucinations (sensory perceptions without sensory input, i.e. hearing voices or seeing something that is not physically there) and delusions (fixed false beliefs, i.e. believing your thoughts are being broadcasted to people around you). Negative symptoms describe a decrease or loss of normal

functioning (i.e. blunted affect, alogia, avolition). A diagnosis of schizophrenia is given if two of the following symptoms are present for a minimum of one month; hallucinations, delusions, disorganized or catatonic behavior and negative symptoms, with enduring signs of functional deterioration (i.e. occupational, social or cognitive dysfunction) present for at least six months (Battle, 2013). In Australia, we typically adopt the DSM-5 as a diagnostic tool, however, the International Classification of Diseases (ICD), Tenth Revision (ICD-10) (International Advisory Group for the Revision of & Behavioural, 2011) is also commonly used to classify and diagnose psychotic disorders. The DSM-5 manual contains >20 pages, whereas the ICD-10 "Bluebook" only contains one page, and hence, the primary differences between the two diagnostic tools lie in the detail specified to meet criteria for schizophrenia. For example, the DSM-5 includes a list of specific sets of symptoms to reference (i.e. 2 or more of Delusions, Hallucinations, Disorganzed speech etc.), and is very specific in that certain symptoms must be present for a significant portion of time with a focus on the impairment in one or more major areas of functioning. In contrast, the ICD-10 delineates "the clinical picture is dominated by relatively stable, often paranoid, delusions, usually accompanied by hallucinations, particularly of the auditory variety…". As opposed to 6 aforementioned core symptoms outlined in the DSM-5, the ICD-10 describes only 3 "common" symptoms: Delusions of persecution, hallucinatory voices, hallucinations of smell or taste.

1.1.2. *Treatment of schizophrenia*

Treatment for schizophrenia has significantly advanced in the last 50 years, with the primary approach being pharmacotherapy in combination with psychotherapy. There is a range of cognitive and sociotherapeutic approaches that are regarded as important factors in the treatment of schizophrenia, such as cognitive behaviour therapy and social and occupational

training. With this said, there is a consensus that these psychotherapeutic treatments are best used in conjunction with a neuroleptic therapy, and this combination is particularly effective during the early illness stages of the disorder (X. Guo et al., 2010). The fortuitous breakthrough in pharmacological treatment for psychosis involved the introduction of the dopamine D2 antagonist chlorpromazine in 1952. Since then, a number of first and second generation antipsychotics have been developed, and this has led to significant improvements in treatment and prognosis for many individuals with schizophrenia. Antipsychotic medications are predominantly effective in reducing the severity of positive symptoms, however, their efficacy is limited in alleviating negative symptoms and cognitive impairments in schizophrenia (Webb & Tandon, 2009).

1.1.3. *Epidemiology of schizophrenia*

Schizophrenia affects between 0.5% to 1.5% of the human population, roughly equating to more than 60 million people worldwide (Maki et al., 2005). In Australia, the prevalence sits at around 1.5%, and in 2010, it was estimated that the annual costs of psychosis on the Australian economy was approximately $4.91 billion, accounting for almost 1.2% of total health expenditure (Neil, Carr, Mihalopoulos, Mackinnon, & Morgan, 2014). These figures do not take into account the tremendous burden and devastating effect schizophrenia has on the lives of those diagnosed and their families. Individuals with schizophrenia typically experience a steep decline in socioeconomic status, with low rates of employment, varying from 12.9% to over 70% (Marwaha et al., 2007) with the predominant source of income being government payment for approximately 85% of individuals with schizophrenia (Morgan et al., 2012). The mortality rate is particularly high, with a 2-3-fold increase relative to the general population, reducing the average life expectancy of individuals with schizophrenia by approximately 20 years (Saha, Chant, & McGrath, 2007).

Schizophrenia can occur at any age, however onset typically peaks during adolescence/early adulthood, between the ages of 15 and 25 in men and 18 and 30 in women (Sutterland et al., 2013). More specifically, in a sample of almost 2000 individuals with schizophrenia, the onset of psychosis occurred under the age of 25 in 65% of cases, between 25-43 years in 23% of cases and between 35-64 years in 12% of cases (Morgan et al., 2012). Although males typically have an earlier onset by approximately 1.5 years, gender differences in the ability to hide or obscure psychotic symptoms may contribute to an earlier diagnosis in males compared to females (Eranti, MacCabe, Bundy, & Murray, 2013). It is also known that women have a second peak in the onset of schizophrenia later in life, after the age of 45, that is hypothesised to be associated with the fall in estrogen associated with menopause (Lindamer, Lohr, Harris, McAdams, & Jeste, 1999).

Data suggest that for the majority of people, psychosis first emerges in late adolescence or early adulthood. Thus, onset commonly coincides with, and impedes a crucial period, during which young individuals' educational, vocational and functional wellbeing is interrupted at a turning point in their developmental trajectory. Although there is significant variability in clinical course, and prognoses may be less detrimental than previously believed (McGorry et al., 2007), outcome in schizophrenia is often typified by a chronic and recurring decline in multiple domains of functioning.

1.2. **The clinical staging model of schizophrenia**

Schizophrenia is a highly heterogeneous disorder, showing large variance across individuals in core psychopathology, treatment response and outcome (Henderson & Malhi, 2014). Complex illnesses such as schizophrenia have been proposed to consist of clinical stages (Agius, Goh, Ulhaq, & McGorry, 2010), where individuals are best considered along an

illness course continuum, as opposed to being in a stable binary state of "ill" or "well" (Agius et al., 2010).

The clinical staging approach to psychiatric disorders was proposed by McGorry et al., (2007) and contrasts conventional diagnostic strategies in that it characterises where a person sits along the continuum of the course of an illness in addition to extent of progression of a disorder at a particular point in time (McGorry et al., 2007). Within this model, early and milder clinical phenomena are differentiated from illness chronicity and progression. Subsequently, there is great advantage in adopting this view for treating young individuals with psychosis, encouraging and enabling the clinician to select treatments relevant to earlier stages of the illness as opposed to a one size fits all approach to therapy. Early psychosis pathology is found to be milder and treatment is often more effective in comparison to later illness stages, when schizophrenia is more established, and the neurobiological abnormalities are more severe (McGorry et al., 2007; Wood, Yung, McGorry, & Pantelis, 2011). Therefore, a clinical staging view of schizophrenia that maps the relationship of biological markers to stage of illness may help to differentiate intrinsic biological processes from epiphenomena and sequelae, and enable existing knowledge to be better represented and understood (McGorry, Killackey, & Yung, 2008). Although the illness course of schizophrenia is not always a linear or a uniform trajectory, it has been proposed that the development of the disorder can be described in at least three clinical stages: ultra-high risk, the first-episode and the chronic phase (Agius et al., 2010).

1.2.1. Ultra-high risk of psychosis

The prodromal period is identified in retrospect, and is characterised by a stage that precedes the onset of schizophrenia. Typically, this period involves subthreshold psychotic symptoms

that affect thought processes, perception, and an impairment in cognition and general functioning (e.g., mood, motivation and social withdrawal etc.). However, since the term "prodrome" can only be definitively applied once psychosis has emerged, the name "ultra-high risk" (UHR) has been termed to imply that psychosis is not an inevitable outcome. Individuals classified as UHR do not always present with subthreshold symptoms, and may meet criteria based on family history. Inclusion in this group requires the presence of either (a) attenuated psychotic symptoms (APS), (b) brief limited intermittent psychotic symptoms (BLIPS), and/or (c) genetic risk (i.e. first degree relative with a psychotic disorder or schizotypal personality) together with functional deterioration (Fusar-Poli et al., 2013; A. R. Yung & McGorry, 1996; Alison R. Yung et al., 2003; Alison R. Yung, Phillips, Yuen, & McGorry, 2004). A recent meta-analysis showed that less than 30% of individuals at UHR will transition to psychosis within a 3-year period (Clark, Schubert, & Baune, 2015).

1.2.2. The first-episode of psychosis

The onset and establishment of psychotic symptoms that are above subthreshold levels signifies the beginning of the first-episode of psychosis (FEP). As previously mentioned, to meet criteria for a psychotic episode, two of the following symptoms must be present for a minimum of one month; hallucinations, delusions, disorganised or catatonic behaviour and negative symptoms, with enduring signs of functional deterioration (i.e. occupational, social or cognitive dysfunction) present for at least six months (Battle, 2013). The FEP is a crucial period when early intervention and pharmacological therapy has the potential to alter the typical progressive chronic course of the illness through minimising the duration of untreated psychosis (DUP) (McGorry et al., 2008). It is known that increased DUP is both a marker and independent risk factor for poor outcome in psychosis (McGorry et al., 2008). Studies have shown that reducing DUP can lead to early benefits, such as reducing illness severity at initial

treatment and suicidal risk, and prolonged benefits, such as improving negative symptoms and social functioning (Johannessen et al., 2005). The relationship between DUP and outcome is robust, and is shown to be sustained over many years of follow-up (Bottlender, 2006), further emphasising the value of accessible treatment and quality during the early illness stage.

The FEP usually occurs during adolescence or early adulthood, a time when the brain is undergoing immense maturational development and change (Shaw et al., 2008). Although psychotic disorders are rare before the age of 14 years, there is a sharp increase in their emergence between 15 and 17 years of age (Kessler et al., 2007) and on average, 50% of individuals who experience a FEP will be in their early 20s (Kessler et al., 2007; Ochoa, Usall, Cobo, Labad, & Kulkarni, 2012). In adolescents experiencing their FEP, the normal neural developmental trajectory has been shown to be altered, with reports of reduced cortical thickness and volumetric reductions relative to healthy controls (Andreasen et al., 2011; Bodnar et al., 2014; Cannon et al., 2015; Galderisi, Merlotti, & Mucci, 2015; Liberg, Rahm, Panayiotou, & Pantelis, 2016; Pantelis et al., 2009). It is estimated that between 10-20% of FEP patients will go on to never experience a recurrence after a single psychotic episode (Cohen, 1995), however the majority of FEP individuals experience a long clinical course involving recurring psychotic episodes, perhaps with periods of remission. Further, some extremely poor-outcome patients may not respond to medication and experience a chronic form of unremitting schizophrenia. This severe form of schizophrenia is known as treatment-resistant schizophrenia (TRS), and affects up to one third of schizophrenia patients and will be one of the focal points of this thesis.

1.2.3. *Treatment-resistant schizophrenia*

The introduction of first and second generation antipsychotics revolutionised treatment in psychiatry, and saw significant improvements in treatment response and quality of life for many individuals with schizophrenia. However, for up to one third of affected individuals, symptoms continue to persist, despite trialing various different types of antipsychotic medications. Consequently, symptomatology is more severe, duration of illness is longer and prognosis is far worse for these individuals (K. Black et al., 2001). Clozapine is a second generation atypical antipsychotic that is a broad-acting antagonist targeting serotonergic alpha adrenergic, acetylcholine and NMDA receptors (Elkis, 2007), and was first introduced into clinical practice in the 1950's (Crilly, 2007). Currently, clozapine is the only antipsychotic that has been found effective in ameliorating psychotic symptoms in individuals who have failed to respond to first-line medications that function principally as dopamine antagonists with additional antagonist properties against the cholinergic, serotonergic and adrenergic systems (Heinz & Schlagenhauf, 2010). Although often effective in treating florid positive symptoms, there are a number of serious and sometimes fatal side effects associated with clozapine, such as myocarditis that can lead to cardiac failure. Further, clozapine is effective in only a percentage of schizophrenia patients, and up to 70% will not respond to what is considered a treatment 'of last resort' (Kane, Honigfeld, Singer, & Meltzer, 1988). These individuals have been termed "treatment resistant". Treatment-resistant schizophrenia (TRS) is one of the greatest therapeutic challenges for clinicians, with patients often suffering an acute and unremitting form of the disorder (Bolonna & Kerwin, 2005).

1.2.3.1. *Definition of Treatment Resistance*

The leading definition of TRS involves three broad criteria (Kane et al., 1988):

(1) The individual must fail to respond to at least two or more adequate trials of antipsychotic

treatment with medications from at least two distinct classes within the last 5 years.
(2) The individual must be experiencing at least two of the following critical psychosis symptoms with moderate to severe acuteness: disorganisation, hallucinatory behaviour, unusual thought content and suspiciousness.
(3) The individual is experiencing significant current symptoms despite antipsychotic treatment, defined as a total Brief Psychiatric Rating Scale (BPRS) score > 45 or a Positive and Negative Syndrome Scale (PANSS) total score > 90.
The relationship between treatment-responsive schizophrenia and TRS is complex, and is not entirely understood. No known pathophysiological characteristic distinguishes TRS from non-TRS, and the only clinical differences seem to be the persistence of severe symptomatology despite pharmacological, psychological and psychosocial therapy. It has been proposed that TRS can be considered one extreme end of the psychosis spectrum, representing the most severe and enduring form of schizophrenia. As such, research aimed at understanding the neurobiological underpinnings that differentiate TRS from non-TRS patients is vital, given at present, there are no available therapies that are effective in treating this acute group.

Taken together, it is well understood that schizophrenia exacts significant personal, familial and societal costs. It is perhaps unsurprising that an expansive research effort has endeavored to uncover the mechanisms underlying this disorder, namely the precipitating and perpetuating factors that drive illness onset and progression to later illness stages.

1.3. **Etiology and pathophysiology of schizophrenia**

Although the etiology of schizophrenia is not completely understood, it is generally agreed that genetic and physiological processes interact with environmental factors to precipitate the

onset of psychosis (Dean & Murray, 2005). It is known that schizophrenia is a highly heritable disorder, and therefore genetic contribution plays a large etiological role. Twin studies have been fundamental in demonstrating the genetic association, with the risk of a monozygotic twin of an individual with schizophrenia also developing the disorder being estimated as 40% to 50% (Narayan, Shikha, & Shekhar, 2015). The genetic architecture of schizophrenia is complex and is yet to be extensively characterised. Research has shown that the disorder is polygenic, polyallelic and common variants of genes identified thus far are not deterministic and only explain part of the genetic risk associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Nevertheless, studying biological unaffected relatives of the disorder can offer valuable insight into the heritable pathophysiology of the disorder independent of factors that often confound studies in patients, such as illness progression and chronic antipsychotic use, and this will be a key focus of this thesis. Furthermore, in addition to identifying heritable risk factors associated with schizophrenia, studying unaffected biological relatives of the disorder can inform *protective factors* through identifying markers that may confer resilience against developing the disorder. Although first-degree biological relatives are considered as being at genetic risk of psychosis (McGorry et al., 2007), typically this population when examined in this context are older (i.e. > 30 years) and have passed the phase of greatest risk for development of a psychotic order (i.e. 15-30 years).

1.3.1. *Neuropathophysiology of schizophrenia*

Schizophrenia is considered a disorder of ‘brain’ and ‘mind’. Psychosis has been present in society for many centuries, and as a result, we know a significant amount about the clinical presentation, onset and response to interventions associated with the illness. More recently, the field has attempted to explain the link between altered brain structure and function and

the clinical features of schizophrenia. The birth of magnetic resonance imaging (MRI) almost 40 years ago saw an explosion in studies that allowed a unique in-vivo way to explore the neurobiological underpinnings of schizophrenia. It has now been widely researched and accepted that schizophrenia is associated with various structural brain abnormalities that in line with the clinical staging model, vary according to illness stage. During the FEP, meta-analyses of structural MRI studies (Ellison-Wright, Glahn, Laird, Thelen, & Bullmore, 2008; Steen, Mull, McClure, Hamer, & Lieberman, 2006; Vita, De Peri, Silenzi, & Dieci, 2006) have repeatedly shown grey matter volume reductions in the whole brain, the prefrontal cortex (PFC), the temporal regions (in particular the superior temporal gyrus), and subcortical structures such as the caudate nucleus relative to healthy controls.

Structural abnormalities appear to be more pronounced, widespread and putatively progressive in chronic schizophrenia groups, with meta-analyses consistently showing volumetric reductions in the aforementioned brain regions, in addition to areas including the hippocampus, amygdala, other frontal and temporal regions such as the orbitofrontal cortex and medial and inferior temporal gyri respectively (Lawrie & Abukmeil, 1998; Shenton, Dickey, Frumin, & McCarley, 2001; Wright et al., 2000). A recent meta-analysis found established schizophrenia patients (n=3547) had 2.6% smaller total brain volumes relative to healthy controls (Haijma et al., 2013).

Given brain structure is highly heritable and largely influenced by genetic variants (Peper, Brouwer, Boomsma, Kahn, & Hulshoff Pol, 2007), studies have investigated the similarities and differences in cortical volume and morphology in unaffected relatives of individuals with schizophrenia. Interestingly, structural brain abnormalities have also been found to be evident in unaffected family members relative to unrelated healthy controls. A recent meta-analysis

found that unaffected relatives (n=584) demonstrated gray matter volume reductions in some, but not all of the areas found to be abnormal in schizophrenia patients (n=495) (Xiao, Zhang, Lui, Yao, & Gong, 2013). Specifically, both patients and unaffected relatives showed reduced grey matter in the left claustrum, the right parahippocampal gyrus, and increased grey matter in the right fusiform gyrus (Xiao et al., 2013). This suggests that a proportion of the structural abnormalities associated with schizophrenia are related to genetic susceptibility to the disorder, indicating that abnormal brain structure cannot be solely the result of illness and medication effects.

As the clinical staging model suggests, pathological deficits vary in severity depending on illness stage. Although there is considerable overlap in the structural abnormalities observed during early and late psychosis, and even in individuals at genetic high risk of developing the disorder, it is extremely worthwhile to investigate these groups separately. Doing so will increase our understanding of the biological risk and protective factors that may be influencing progression from one illness stage to the next.

1.3.2 *Functional magnetic resonance imaging (fMRI) and network connectivity in schizophrenia*

It is well understood that various structural abnormalities are associated with various illness stages of psychosis, however, the complexity and heterogeneity of disruptions to various neural processes in schizophrenia suggests the disorder is unlikely a result of isolated morphological disruptions affecting a single or handful of brain regions. Instead, research favours current theories of a more distributed pathogenesis affecting communication within and between brain systems and networks. A number of tools have been developed to explore the neurobiological mechanisms underlying the onset and establishment of schizophrenia.

Recent advances have gone beyond investigating structural and morphological properties such as volume, surface area and cortical thickness to investigate structural and functional connectivity between brain regions. The methodological approaches I will focus on for the purpose of this thesis are: (1) resting-state functional connectivity, which measures the temporal co-activation of spatially distinct brain regions when the brain is not engaged in any specific task, and (2) functional network topology, which uses graph theory measures to understand the intrinsic architecture of brain networks. A brain networks functional topology can be measured using various computations, and for the purpose of this thesis I will focus on three in particular; global and local efficiency, and small worldness. For further explanation and definitions of the fMRI methods used in this thesis, please refer to the general methodology section on page 49.

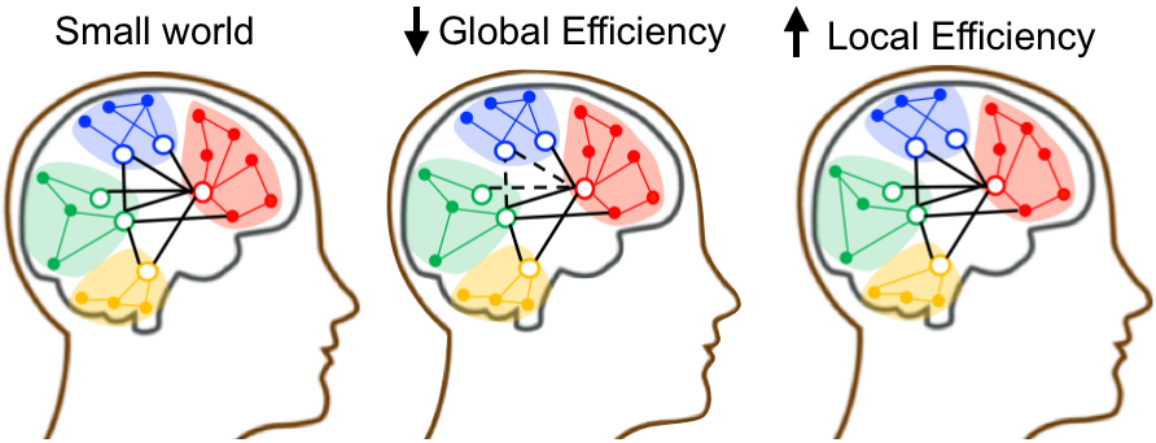
1.3.3. *The disconnection hypothesis of schizophrenia*

The disconnection hypothesis was introduced more than 20 years ago (K. J. Friston & Frith, 1995; Weinberger, 1993), and postulated that schizophrenia is best understood as a disorder of brain "dysconnectivity". The brain is believed to be a complex distributed network of regions that interact and work synergistically to integrate and process sensory, cognitive and emotional information (Brennan, Harris, & Williams, 2013). The disconnection hypothesis suggests that this system is disturbed in schizophrenia, resulting in aberrant integration and communication of information between brain regions (K. Friston, Brown, Siemerkus, & Stephan, 2016), that can lead to a misinterpretation of the external and internal world. Since its inception, the advent of functional MRI (fMRI) has facilitated the investigation and supported the notion of dysconnectivity in schizophrenia.

1.3.4. *Is small-worldness altered in schizophrenia?*

The current consensus is that the human brain demonstrates small-world properties that support high global and local efficiency to maintain a balance between local processing speed and global integration of information (M. Li, Chen, & Li, 2012). Is this basic architecture disrupted in schizophrenia? Small-world network properties appear to be maintained in individuals with schizophrenia, however specific topological metrics have been found to be disturbed (He et al., 2012; Y. Liu et al., 2008; Lynall et al., 2010). Often, reduced local and global efficiency is reported in schizophrenia, suggesting a disrupted balance between local processing and global integration that may result in delayed communication between brain regions, and reduced ability to integrate information (M. Li et al., 2012). Conversely, some studies have reported increased network efficiency in schizophrenia, suggesting a shift towards topological randomisation relative to healthy controls (Alexander-Bloch et al., 2010; Y. Liu et al., 2008; Lynall et al., 2010). See Figure 1.1 for a schematic representation of decreased global and increased local efficiency that has frequently been reported in schizophrenia cohorts. The application of graph theoretical measures to understand functional network topology in psychiatric illnesses such as schizophrenia is in its infancy stage, and further research in this area is critical to complement and potentially further elucidate the currently heterogeneous resting-state functional connectivity (rs-FC) findings.

Figure 1.1. Schematic representation of small world brain network configuration and how this can be disturbed in schizophrenia. The solid coloured circles represent nodes and the unfilled circles represent hub nodes (which enable long-range connections from one module to another). The coloured lines represent intra-modular connections and the black lines represent long-range inter-modular connections.



1.3.5. *Resting-state functional network connectivity and topology in schizophrenia*

As previously mentioned, functional disconnection has been proposed as a key etiologic factor in schizophrenia (K. J. Friston & Frith, 1995; Weinberger, 1993), and accordingly, rs-FC has provided many valuable insights into the functional network abnormalities that are associated with different illness stages of schizophrenia. Adopting a staging approach to psychosis neuroimaging research creates a framework to map pathophysiology to stages of the disorder, and in turn distinguish core biological processes from epiphenomena and sequelae (McGorry et al., 2007). For the purpose of this thesis, I will now discuss the recent advances and gaps in the resting-state functional neuroimaging field of three different populations associated with schizophrenia: 1) first-episode psychosis (FEP); 2) treatment-resistant schizophrenia (TRS) and 3) unaffected family members (UFM) of individuals with schizophrenia.

1.4. Resting-state functional connectivity in first-episode psychosis (FEP)

As previously discussed, there has been a shift in the last 5-10 years to explore network abnormalities that may be present in the early illness phase of psychosis, with the aim of

characterising neurobiological factors that could be contributing to the onset and establishment of schizophrenia. As a result, a breadth of research has been conducted exploring abnormal rs-FC in FEP cohorts, however results are quite divergent, partially due to the widely-varied methods used across research groups (Greicius, 2008). To broadly summarise previous research and attempt to infer meaning from results, the various methodology used can be categorised into two general approaches; seed-based rs-FC methods or data driven whole-brain methods.

1.4.1. *Seed-based rs-FC studies in FEP*

The majority of rs-FC analyses conducted in FEP populations over the past 5 years have adopted the seed-based approach, and results reported are highly mixed. The seed-based approach involves defining a seed, or region of interest (ROI), such as the insula. The mean time series for that region is then obtained by averaging the time series of all voxels comprising the insula. Following this, the correlation between the ROI (e.g. insula) and all other grey matter voxels of the brain is computed, resulting in a comprehensive spatial correlation map of the whole brain (Smith et al., 2013) (see general methodology page 51 for further explanation). Relative to healthy controls, FEP individuals have shown increased rs-FC (Bao et al., 2016; Cui et al., 2015; Ebisch et al., 2014; Fornito et al., 2013; Gong et al., 2016; W. Guo et al., 2015; W. Guo, Yao, et al., 2014; T. Li et al., 2016; Yoon et al., 2015), and decreased rs-FC (Alonso-Solis et al., 2012; Anticevic et al., 2014; Bastos-Leite et al., 2015; Cui et al., 2015; Ebisch et al., 2014; Fornito et al., 2013; Gong et al., 2016; W. Guo et al., 2015; W. Guo, Xiao, et al., 2014; W. Guo, Yao, et al., 2014; X. Guo et al., 2013; T. Li et al., 2016; Sole-Padulles et al., 2016; Yoon et al., 2015). No known study to date has reported no significant rs-FC differences between FEP individuals and healthy controls.

Few studies overlapped in the ROI specified, and therefore, almost all rs-FC abnormalities reported are yet to be replicated. Although there has been little consistency in ROI's investigated across multiple studies, there was some overlap in frontal ROIs, such as the dorsolateral and medial prefrontal cortex (dlPFC and mPFC) (Cui et al., 2015), and the prefrontal cortex broadly (Anticevic et al., 2015). Anticevic et al., (2015) reported increased rs-FC in early course schizophrenia patients within prefrontal regions, particularly between the PFC and inferior and middle frontal cortex relative to healthy controls at baseline. At 12-months follow-up, PFC hyperconnectivity had normalised for a subset of the sample, and this normalisation predicted symptom improvement. Cui et al., (2015) found FEP patients to show increased rs-FC between anterior cingulate cortico (ACC)-prefrontal-hippocampal regions and reduced rs-FC between ACC-related and hippocampal-dorsolateral prefrontal-medial regions, and no relationship between rs-FC and symptomatology in FEP. Taken together, these findings indicate that FEP individuals exhibit predominantly increased rs-FC involving frontal regions. This finding of hyper-connectivity involving frontal regions differs to what is observed in established schizophrenia cohorts, indicating that something is happening during this early illness stage which may be interrupting normal brain development.

1.4.2. *Whole-brain rs-FC studies in FEP*

Relative to studies that adopted a seed-based approach, substantially fewer research groups have used a whole-brain approach to explore rs-FC, with only five known studies employing data driven hypothesis-free methods (Argyelan et al., 2015; W. Guo et al., 2017; W. Guo, Xiao, et al., 2014; T. Li et al., 2016). Whole-brain rs-FC commonly involves anatomically partitioning the brain into distinct regions, often with the application of an established brain parcellation template that has functionally subdivided the cortex. The Pearson correlation is

then calculated between each possible pair of regions to determine a measure of functional connectivity (see general methodology page 50 for further explanation). Three of these studies investigated inter-hemispheric rs-FC which involved calculating the Pearson correlation coefficient between each voxel's time series and that of its interhemispheric mirrored voxel. The first study by Guo & Xiao, et al., (2014) found reduced interhemispheric rs-FC in drug naïve FEP participants within the precuneus, precentral gyrus, superior temporal gyrus (STG), middle occipital gyrus and fusiform gyrus relative to controls. There was no evidence of increased rs-FC in FEP (W. Guo, Xiao, et al., 2014).

Li and colleagues (2015), later reported reduced interhemispheric rs-FC in the STG and postcentral gyrus in a group of younger, early onset schizophrenia (EOS) patients that were all drug naïve. Most recently, Guo et al., (2017) compared interhemispheric rs-FC in drug naïve FEP participants, and found reduced interhemispheric rs-FC in the precuneus in FEP participants relative to controls (W. Guo et al., 2017). Taken together, these studies suggest that rs-FC (specifically interhemispheric rs-FC) is reduced in FEP relative to healthy controls, and in particular, regions such as the precuneus, superior temporal gyrus, and fusiform gyrus appear to be implicated across multiple studies. Importantly, these findings of reduced rs-FC were evident in drug naïve FEP samples, suggesting they are not confounded by the effects of antipsychotic medication.

Other studies that investigated whole-brain rs-FC in FEP showed reduced rs-FC across multiple resting-state networks (Argyelan et al., 2015). Conversely, Li et al., (2016) observed increased rs-FC between various temporal, frontal and subcortical regions (T. Li et al., 2016). Additionally, Li et al., (2016) found reduced rs-FC between parietal and frontal regions in FEP participants relative to controls, that did not significantly correlate with symptom

severity. Increased rs-FC was not reported by any of the aforementioned whole-brain FEP studies, with all significant findings reporting reduced rs-FC in FEP relative to controls. Inconsistency in direction of findings (i.e. increase vs. decreased rs-FC) between ROI and whole brain studies may be related to the timing of scan in relation to proximity of illness onset, and the brain's response to this. Seed-based studies had relatively short average illness duration of the FEP group (<12-months) compared to other whole-brain studies that have mean illness durations ranging from 19 to 24 months. It has been proposed that early psychosis can be associated with a compensatory neurofunctional response to neuroanatomical deficits (W. Guo et al., 2013). As such, there can be a trade-off within the brain to compensate for anatomical or functional deficits to prevent global brain performance from further decline (Cabeza, Anderson, Locantore, & McIntosh, 2002). An alternative theory is that hyperconnectivity results from a dedifferentiation, namely a generalised increase in functional connectivity (Logan, Sanders, Snyder, Morris, & Buckner, 2002), however direct evidence of these hypotheses is not available. Of the few whole-brain studies, the majority restricted analyses to explore inter-hemispheric rs-FC or rs-FC within specific resting-state networks, once again limiting the brain wide inferences that can be made. In order to further disentangle potential network abnormalities that have occurred, or are occurring during the early illness stage of psychosis, there is a need for more comprehensive data-driven approaches to interrogating rs-FC.

1.4.3. *Rs-FC and symptomatology in FEP*

Of the 20 studies that have investigated rs-FC in FEP over the past 5 years, 10 reported significant associations between abnormal rs-FC and symptomatology. Of these 10 studies, 4 reported an association between reduced rs-FC and symptomatology in FEP (Anticevic et al., 2015; W. Guo, Xiao, et al., 2014; H. J. Li, Xu, Zhang, Hoptman, & Zuo, 2015; Y. Wang et

al., 2017). These studies predominantly found relationships between reduced frontal and subcortical rs-FC (i.e. orbitofrontal cortex, amygdala and precentral gyrus) and negative and/or total symptomatology. Conversely, the remaining 6 studies reported associations between increased rs-FC and symptom severity in FEP (Ebisch et al., 2014; Fornito et al., 2013; T. Li et al., 2016; Tang et al., 2013; S. Wang et al., 2017; Zhou, Tan, Tang, & Chen, 2010). Increased rs-FC involving frontal regions, such as, prefrontal, medial frontal and inferior frontal areas in addition to subcortical regions including the posterior cingulate cortex and insula were found to be associated with symptomatology in FEP groups, with positive symptom severity most frequently implicated. Although the literature is somewhat divided as to whether increased or decreased rs-FC has a modulatory effect on symptomatology, results indicated that perhaps reduced rs-FC is more associated with negative symptom severity, and in contrast, increased rs-FC is more strongly correlated with positive symptomatology. Regardless of the direction of rs-FC reported, the literature suggests that there is evidence of functional connectivity disturbances in FEP, and in some cohorts, abnormal rs-FC involving frontal regions is associated with symptom severity.

1.4.4. *Why are results in FEP so mixed?*

Participants with FEP display immense heterogeneity in their symptom expression and long-term outcome. Acute symptoms may oscillate and new affective and psychotic symptoms might emerge. Therefore, an initial diagnosis within the psychosis spectrum is not stable, and as such, neurobiological correlates of the disorder will vary greatly across studies depending on patient group characteristics and outcome. The mixed literature may also be due to a number of other factors, such as widely varying methodologies used to map rs-FC, underpowered sample sizes and inherent heterogeneity in patient characteristics and demographics. For example, previously studied FEP samples comprise participants with ages

spanning from adolescence to adulthood (15-33 years of age), and duration of illness ranging from weeks (e.g. 0.27 months) (T. Li et al., 2016), to years (e.g. 24 months) (W. Guo et al., 2017). Consequently, moderating factors such as age, duration of illness and medication use are highly varied across studies, all of which have been shown to have an effect on rs-FC (Andreasen et al., 2011; Sarpal et al., 2017; Sarpal et al., 2015; X. Wang et al., 2014). Alternatively, it is possible that the heterogeneity of findings at the earlier stages of illness reflect differing stages of progressive structural brain changes on a background of dynamic maturational brain changes (Cropley & Pantelis, 2014; Gogtay, Vyas, Testa, Wood, & Pantelis, 2011; Pantelis et al., 2009). Thus, mapping brain function longitudinally presents an important way forward. Whether changes in rs-FC are progressive or not remains unclear, with studies that have investigated rs-FC over time concentrating on pre-and post-antipsychotic administration in FEP. In order to better characterise factors that could be contributing to the onset and establishment of schizophrenia, we require a more comprehensive understanding of the neurobiological differences that are present during the early illness phase, and how they develop over time. In light of the highly-varied literature, perhaps the classic approaches of characterizing rs-FC cross-sectionally are not sensitive or specific enough to address this question.

1.4.5. *Rs-FC in FEP over time*

The few studies that have investigated longitudinal changes in rs-FC in FEP have concentrated on functional brain changes in drug naïve individuals pre-and post-antipsychotic administration (Abbott, Jaramillo, Wilcox, & Hamilton, 2013; Lui et al., 2010; Sambataro et al., 2010; Sarpal et al., 2015). The duration between visits were brief and varied across studies, ranging from 28 days (Sambataro et al., 2010) to 12-weeks (Sarpal et al., 2015). Results indicate that some, but not all rs-FC abnormalities that are present during the early

illness phase normalise after initial treatment with antipsychotic medication, suggesting that both state and trait neural correlates of the disorder may be evident during the early illness stage. It would be of interest to extend this literature and explore rs-FC changes longitudinally in a FEP cohort that is already on an established treatment regime to further differentiate state from trait network abnormalities associated with FEP.

1.4.6. *Network topology in FEP*

In addition to employing a longitudinal design, another way to enhance our understanding of the neurobiological functional differences and changes that are present during the FEP illness phase, and which may help explain the inconsistent rs-FC findings, is by broadening our scope to explore other features of connectivity using graph theory. The advantage of such an approach is that it goes beyond connectivity analyses and facilitates the exploration of complex networks and metrics, such as efficiency of information transfer on whole brain and regional scales (Rubinov & Sporns, 2010). Recently, in conjunction with measures of functional connectivity, graph theoretical methods have been applied to fMRI data in an attempt to understand the topology and efficiency of brain networks. Previous studies have reported abnormal structural (Fornito, Harrison, Zalesky, & Simons, 2012; Zalesky et al., 2011) and functional brain network topology in established schizophrenia (Hadley et al., 2016; Lo et al., 2015; Lynall et al., 2010), however, few studies have used graph theory to understand network organization in FEP. While structural network topology has been found to be abnormal in early illness stages (Crossley et al., 2017; Hu et al., 2016; Palaniyappan et al., 2016; Zhang et al., 2015), to our knowledge, no study has examined functional network topology during resting-state in FEP longitudinally. Further, investigating functional brain networks in psychosis using graph-theoretic methods may better elucidate the interplay

between network arrangement and communication during the early stages of the disorder, and longitudinally.

1.5. **Neuropathophysiology of treatment-resistant schizophrenia (TRS)**

Structural abnormalities in brain anatomy have been observed in TRS patients relative to treatment responsive patients, such as reduced grey matter in frontal areas (Anderson, Goldstein, Kydd, & Russell, 2015; Kubera et al., 2014; Mitelman, Shihabuddin, Brickman, Hazlett, & Buchsbaum, 2005; Quarantelli et al., 2014; Zugman et al., 2013), and enlarged white matter volumes (Molina et al., 2008). However, given the complexity and severity of this potential subgroup/illness stage of schizophrenia, the underlying pathology that is perpetuating resistance to pharmacotherapy is unlikely a result of a regional grey matter disruption in isolation, but is more likely due to a host of brain regions that are abnormally communicating and integrating information.

Despite the clinical relevance of TRS, few functional neuroimaging studies have focused on this population at a network level. A central goal of personalised medicine in psychiatry is to predict an individual's response to specific therapies. An important step towards achieving this in TRS is to comprehensively understand the pathophysiology of this proposed subtype of schizophrenia. Further elucidating the neurobiology that is associated with TRS will in turn improve our understanding of the disorder, and hopefully progress towards the development and implementation of effective alternative treatments. Furthermore, characterising the neural networks associated with TRS could facilitate future machine learning research that is aimed at predicting patient response to treatment at the individual level.

1.5.1. *Rs-FC in TRS*

There have been five known studies that used resting-state fMRI to investigate functional connectivity in individuals with TRS. Four of the five studies investigated rs-FC in TRS patients relative to healthy controls, and all found significant differences in functional connectivity in the TRS group. The first, by Vercammen and colleagues (2010) used a seed-based approach to show TRS individuals with auditory verbal hallucinations (AVH) to have *reduced* rs-FC between the left superior temporal gyrus and right Broca's area relative to controls. The severity of AVH in the TRS group negatively correlated with rs-FC between the left temporoparietal junction and bilateral anterior cingulate cortex and amygdala. Conversely, Wolf et al., (2011) found both *increased* and *decreased* rs-FC in TRS relative to controls. Specifically, the TRS group showed *increased* rs-FC in the bilateral temporal lobes, frontoparietal resting-state network (RSN) (the right middle frontal gyrus in particular), the executive control RSN (specifically, the right middle frontal gyrus and superior frontal gyrus), and *reduced* rs-FC in the cingulate cortex and the left precuneus in the executive control RSN, relative to controls. Wang et al., (2015) found abnormal rs-FC in the TRS group predominantly involving prefrontal and temporal regions relative to healthy controls.

Only two known studies have investigated rs-FC in TRS versus treatment responsive schizophrenia patients. Alonso-Solis and colleagues (2015) found increased rs-FC between the dorsomedial prefrontal cortex and other frontotemporal regions, and reduced rs-FC between the ventromedial prefrontal cortex and the cingulate cortex in TRS patients relative to treatment-responsive schizophrenia patients (Alonso-Solis et al., 2015). The most recent study by White et al., (2016) compared TRS patients with non-refractory schizophrenia patients (patients that did not satisfy TRS criteria), and found that individuals with TRS had

reduced rs-FC between the ventral striatum and substantia nigra relative to treatment-refractory patients.

Despite each neuroimaging study conducted in TRS reporting significant differences in rs-FC relative to both controls treatment-responsive schizophrenia patients, findings are divergent in the regions implicated and the direction of abnormal rs-FC reported. Taken together, brain regions consistently found to show abnormal rs-FC across multiple studies in TRS are temporal and prefrontal structures, particularly the cingulate cortex, medial prefrontal cortex and the superior temporal gyrus.

1.5.2. *Rs-FC and symptomatology in TRS*

Of the five known studies that investigated rs-FC in TRS, three reported significant associations between abnormal rs-FC and symptom severity (Vercammen, Knegtering, den Boer, Liemburg, & Aleman, 2010; J. Wang et al., 2015; White et al., 2016; Wolf et al., 2011). Two studies found significant relationships between auditory verbal hallucinations and temporal rs-FC, specifically, reduced rs-FC between the temporoparietal junction and the ACC and amygdala (Vercammen et al., 2010), and increased rs-FC within superior temporal gyrus (Wolf et al., 2011). The remaining study found a significant association between increased positive symptom severity and reduced ventral striatum and cerebellar rs-FC (White et al., 2016).

1.5.3. *Why are results in TRS so mixed?*

Structural MRI studies uniformly show reduced grey matter in TRS patients relative to healthy controls, and this is consistent with findings reported in the general schizophrenia population (Mouchlianitis, McCutcheon, & Howes, 2016). Rs-FC findings are not as

homogeneous, and this variance is also seen in broad schizophrenia cohorts. Heterogeneity of results may be in part due to the diverse methods used, with only one study having adopted a data driven whole brain approach. When different studies adopt a slightly different version of the seed-based method, such as, choosing different brain regions as ROI's or different RSN's as masks, it makes drawing inferences across multiple studies difficult. Further to this, the TRS cohorts varied greatly in the patient characteristics across studies. For example, illness duration ranged between an average of 10 and 17 years, and exposure to antipsychotic medication highly differed, with some/all TRS participants on antipsychotics with varying dosage, drug class, and combination of medications. Both illness duration and antipsychotic medication have been found to have an effect on rs-FC (Sarpal et al., 2017; Sarpal et al., 2015; X. Wang et al., 2014). Further, the variable definition of treatment resistance used across studies may contribute to heterogeneity of results, given some studies classify a cohort as TRS simply if they experience persistent positive symptoms. Additionally, mixed results might suggest that rs-FC alone is not sensitive or specific enough to identify network properties that are specific TRS or to schizophrenia in general. To date, no study has investigated graph theoretical measures of functional network topology in a TRS group, and considering the limited and mixed literature, it seems a logical next step. Therefore, the use of standardised criteria to define TRS, and more data-driven approaches to methodology that go beyond looking at rs-FC alone, to explore network topology in conjunction with rs-FC would maximise inference and further elucidate the neurobiology underlying TRS.

1.6. **Investigating unaffected family members (UFM) of individuals with schizophrenia**

The traditional case-control design is a powerful tool to detect neurobiological abnormalities that are present in individuals with schizophrenia relative to the general healthy population.

However, this approach is often confounded by factors such as clinical and treatment histories of patients that limit the inferences that can be made surrounding the etiology of schizophrenia. It is thus often difficult to ascertain whether brain abnormalities identified in patients relative to controls result from state illness and medication effects, or are more attributed to inherent genetic risk endophenotypes (Moran, Hulshoff Pol, & Gogtay, 2013). Considering schizophrenia has a strong genetic component, studying unaffected biological relatives can provide insight into heritable trait characteristics associated with the disorder, and in turn identify putative endophenotypes of schizophrenia. In terms of the clinical staging model, although unaffected family members are considered as being at 'low risk' of psychotic illness, typically the cohorts studied are older than the average age of onset.

1.6.1. *Endophenotypes in schizophrenia*

Endophenotypes in psychiatry are measurable, trait-related, heritable characteristics that are illness-associated and over-represented in unaffected relatives of probands in comparison to the general population (Gottesman & Gould, 2003). Over the past 5 years, endophenotype research in schizophrenia has identified a number of anatomical risk markers associated with the disorder. A number of structural morphological alterations have been found in UFM compared to healthy controls, including cortical thinning (Gogtay et al., 2007; Gogtay et al., 2003), whole brain (McIntosh et al., 2011; Thermenos et al., 2013) and subcortical volume reductions (Thermenos et al., 2013), with abnormalities usually appearing less severe relative to schizophrenia patients. Given the field of schizophrenia research is continuing to move towards pathophysiological models that particularly involve neural abnormalities at the network level as opposed to the regional level, research into functional network properties as potential trait related endophenotypes of schizophrenia is in its early phase.

Alternatively, rs-FC alterations that are unique to UFM *and* absent or moderated in affected relatives and the general population might be hypothesised to represent putative markers of resilience to schizophrenia, and counterbalance any familial liability for the disorder. Biological markers of resilience have not been extensively studied in schizophrenia, with resilience in psychiatry traditionally discussed in terms of psychological response to stress and trauma (Feder, Nestler, & Charney, 2009; Russo, Murrough, Han, Charney, & Nestler, 2012). Recent evidence suggests that brain networks in UFM exhibit increased resilience to pathological disruption, that is, a brain network is more resilient and intact after the removal of a brain region or node relative to schizophrenia patients and healthy controls (Lo et al, 2015). Further, studies have shown that unaffected siblings of patients with childhood-onset schizophrenia can recover from grey matter developmental delays (Chakravarty et al., 2015; Zalesky et al., 2015). Resilience endophenotypes inferred from rs-FC have also been reported in other psychiatric disorders, such as depression (Peterson et al., 2014). These previous studies motivate further investigation of rs-FC brain networks properties that may be associated with resilience in schizophrenia.

1.6.2. *Rs-FC in UFM; whole brain studies*

Only four known studies have adopted a whole-brain data-driven approach to investigating rs-FC in UFM, and findings are mixed. The first known whole-brain study by Liu et al., (2012) used rs-FC pattern classification to show UFM and probands shared altered rs-FC, with a pattern classification separation (i.e. rs-FC differences) of 77.6%, relative to the 80.4% pattern separation between probands and healthy controls, and the 78.7% separation between healthy controls and UFM. Although this study did not specify the brain regions involved nor the direction of shared rs-FC patterns reported, results indicated that probands and UFM have more similar rs-FC profiles, relative to probands vs. controls and UFM vs. controls. This is

somewhat expected given the heritability of network properties such as functional connectivity. Adopting a similar approach, Wang et al., (2015) generated whole brain rs-FC maps for schizophrenia patients, unaffected siblings and healthy controls to investigate similarities and differences across the three groups. Results suggested TRS patients and unaffected siblings shared numerous rs-FC patterns, predominantly involving intra cerebellar connectivity, and rs-FC between the cerebellum and occipital, prefrontal, paracentral and thalamic regions. Wang et al., (2015) also identified a number of sibling specific rs-FC patterns, where patients showed decreased rs-FC and siblings showed increased rs-FC, particularly between occipital and frontal, parietal and temporal regions. This sibling specific rs-FC was hypothesised to potentially indicate a compensatory response to genetic liability, or a marker of network resilience that is present in UFM. Conversely, Lui et al., (2015) found no evidence of rs-FC abnormalities in UFM relative to controls or probands, despite finding abnormal rs-FC in schizophrenia patients relative to healthy controls. This suggests that although rs-FC is to some extent genetically driven and heritable, the network abnormalities observed in this schizophrenia cohort may have resulted from illness and treatment effects, rather than the manifestations of common/familial genetic risk factors (Lui et al., 2015). Most recently, Guo et al., (2017) used a family-based case-control design and found no rs-FC differences in UFM relative to controls. This study did however find reduced interhemispheric rs-FC in FEP patients in the precuneus relative to both controls, and in the fusiform gyrus, lingual gyrus and precuneus relative to UFM. This suggests that UFM and controls do not differ in interhemispheric rs-FC, and if there are shared rs-FC abnormalities between UFM and probands, they are too subtle to be detected/statistically significant in this sample. The observed reduced rs-FC in the FEP patients relative to UFM may be suggestive of a resilience endophenotype in the UFM, however this is speculative, given there were no significant increases in rs-FC in UFM relative to controls.

1.6.3. *Seed-based studies in UFM*

In addition to the abovementioned studies, the majority of rs-FC endophenotype research in UFM adopted a seed-based approach. Although there was little consensus in the ROI chosen, the few studies that did overlap specified temporal (W. Guo et al., 2015; Oertel-Knochel et al., 2013) and prefrontal regions as ROI's. In particular, there was substantial overlap in the choice of the dorsolateral prefrontal cortex (dlPFC) as a seed region (H. Liu et al., 2012; Peeters et al., 2015; Su et al., 2013; Unschuld et al., 2014; Xi et al., 2016). In summary, studies that investigated rs-FC of the dlPFC found UFM to show reduced rs-FC between the dlPFC and temporal and frontal regions relative to controls at intermediate severity (i.e. less severely reduced rs-FC) relative to schizophrenia patients (Peeters et al., 2015; Su et al., 2013). Conversely, Liu et al., (2012) found no significant differences in rs-FC between UFM and controls. Increased rs-FC between the left dlPFC and right inferior frontal gyrus was observed in schizophrenia patients relative to their UFM, however this only approached statistical significance in comparison to healthy controls (H. Liu et al., 2012). Interestingly, despite these studies specifying the same ROI, results varied. This could be due to a number of contributing factors, such as sample heterogeneity and variance in the pre-processing steps and methodology used to analyse seed-based rs-FC. Alternatively, it could be that rs-FC alone is not sensitive or specific enough to detect functional network endophenotypes that may be present in UFM.

Despite variation in the results reported, most whole-brain and seed-based studies show similar although often milder network abnormalities in UFM in comparison to their affected relatives, and most often, the findings have been in the direction of reduced rs-FC (J. Wang et al., 2015). Rs-FC abnormalities in UFM suggest that aberrant network connectivity may be a

marker of genetic vulnerability to schizophrenia as opposed to being solely a result of illness duration, medication and/or other secondary environmental factors.

1.6.4. *Network topology in UFM*

As previously discussed, in an attempt to further understand the intrinsic organisation of functional brain networks, a growing number of studies have applied advanced graph analysis techniques to rs-fMRI. To our knowledge, only one study has investigated functional network topology in UFM of schizophrenia patients. Lo et al., (2015) demonstrated a shift towards topological randomisation in schizophrenia patients, and to a lesser extent in UFM relative to controls. Specifically, UFM showed reduced clustering, increased efficiency and greater resilience to targeted attack (the targeted removal of a node from the network) relative to controls. This finding is yet to be replicated or further investigated and thus, whether this shift towards randomisation in UFM is a heritable schizophrenia resilience endophenotype, remains unclear. Given the high heritability of rs-FC and functional network topology (Fornito & Bullmore, 2012; van den Heuvel et al., 2013), and the high heritability of schizophrenia, further investigation into network connectivity and architecture in UFM and schizophrenia patients is necessary.

1.7. **Rationale and Aims**

The findings reviewed above emphasise the association between disrupted rs-FC and schizophrenia. Although progress has been made in uncovering the biological mechanisms underlying psychosis, there is still much that needs to be done to comprehensively characterise the etiology and pathophysiology of the disorder. It is clear from previous research that there are different illness stages of psychosis, and that these stages entail different pathophysiology and symptom severity. After the first-episode of psychosis, if the

illness progresses, the early years may be characterised by intermittent psychotic episodes and periods of remission following successful treatment cycles. Conversely, poor-outcome patients may experience a chronic form of unremitting schizophrenia and some also may not respond to medication. Therefore, it seems logical to map neurobiological abnormalities across different stages of schizophrenia, and where possible, to map neurobiological abnormalities longitudinally. This approach will further characterise the trajectory of neural deficits associated with the disorder, and in turn, help to differentiate what is precipitating psychosis onset from what is perpetuating or resulting from the establishment of the disorder.

Currently, it is known that rs-FC is disturbed in established schizophrenia, however substantially less research has been conducted in cohorts that are at either ends of the schizophrenia spectrum, namely treatment-resistant schizophrenia (TRS) and first-episode psychosis (FEP). The limited number of studies that adopted a whole-brain, data-driven approach to explore rs-FC in these cohorts means the literature as it stands today is constrained, mixed and largely un-replicated. Further to this, only a handful of studies have probed deeper into functional brain network properties of individuals with schizophrenia to investigate whether functional networks are topologically intact, and literature pertaining to this research question is particularly sparse in TRS and FEP populations. The scarcity of knowledge on these points led to the following research questions in this thesis.

1. Is rs-FC and network topology impaired in the early FEP stage of schizophrenia, and how does rs-FC and network topology change over a 12-month period in FEP relative to healthy controls?
2. Is rs-FC and network topology affected in TRS patient's relative to healthy controls? How does this compare to network properties in the early illness stage of schizophrenia?

3. Is abnormal rs-FC and network topology a potential endophenotype of TRS?

In order to address these questions, three empirical studies were conducted in which both rs-FC and graph theoretic measures were investigated.

1.8. **References**

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1.9. General methodology

1.9.1. Functional magnetic resonance imaging (fMRI)

Functional MRI is a non-invasive neuroimaging technique that measures changes in oxygenated blood flow in the brain. fMRI relies on the principal that cerebral blood flow and neuronal activation are intrinsically linked, that is, when a region of the brain is in use, blood flow to that region will increase (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). Via the hemodynamic response, blood releases oxygen and glucose to active neurons at a greater rate than to inactive neurons, and this gives rise to a measurable change in the local ration of oxy- to deoxyhemoglobin. This contrast of blood deoxyhemoglobin is referred to as the blood-oxygen-level dependent (BOLD) signal, and is a localizable marker of brain activity. MRI technology uses a permanent static magnetic field to align nuclei in the brain, and a gradient magnetic field that spatially locates the different nuclei. Given deoxygenated haemoglobin is more magnetic than oxygenated haemoglobin, fMRI technology uses these differences in magnetic properties to enable the measurement of the BOLD signal.

1.9.2. Functional connectivity

Functional connectivity is defined as the temporal co-activation between spatially distinct brain regions (Lee, Harrison, & Mechelli, 2003), and reflects the amount of communication that occurs between structures. In contrast to anatomical connectivity that describes the physical white-matter connections between brain regions, functional connectivity explores regional interactions in the brain in the form of correlated activation patterns (BOLD signal). Brain regions that have correlated activation patterns are said to be functionally connected, that is, neurons that fire together, wire together (Shatz, 1992). The most commonly adopted measure of determining the functional connectivity between two brain regions is simply calculating the Pearson's correlation between the time series of said regions. A single voxel's

response signal (or BOLD signal) over time is referred to as its timecourse, and for a given brain region, the time series is determined by averaging the timecourse of all voxels that comprise that region. The measure of functional connectivity used in the current thesis does not provide information on the direction of the effect of one brain region on another. That is, it cannot be inferred that brain region A has a causal influence on brain region B. This measure of functional connectivity is known as effective connectivity, and although it can offer unique insight into brain network configuration and organization, the inclusion of the effective connectivity methodology was beyond the scope of this thesis.

1.9.3. Resting-state functional connectivity (rs-FC)

Functional connectivity is often explored in relation to some form of cognitive task that is performed while the subject is inside the scanner, however this can be disadvantageous in clinical populations such as schizophrenia where individuals may not understand or be able to complete the task (Greicius, 2008). Alternatively, resting-state functional connectivity (rs-FC) measures the temporal co-activation of brain regions while the participant is not engaged in any specific task, but is instead instructed to close their eyes, relax, and let their mind wander without falling asleep for approximately 8-10 minutes. Research has shown that spontaneous low-frequency BOLD signal fluctuations during rest show a high degree of temporal correlation across the brain. These patterns of co-activation are robust, reproducible and can be characterized into various resting-state networks related to visual processes, sensorimotor processing, executive functioning, attention, salience and default-mode networks. Rs-FC has the ability to probe the brains intrinsic functional architecture and is free of many confounds associated with task-based fMRI, such as difficulty in task administration, require cognitive aptitude to understand the task and task performance. Rs-FC is thus a popular approach and a valuable tool in understanding brain disorders such as

schizophrenia, and will be a key approach to investigating schizophrenia neuropathophysiology associated with schizophrenia in this thesis.

Motion is an important consideration in fMRI data analysis. Head motion, scanner artefacts and various other noise-related fluctuations can confound rs-FC measures. Although there is no gold standard of denoising steps, the present thesis used a number of well-established motion correction considerations. Head motion was controlled with the Friston 24-parameter model (Friston, Williams, Howard, Frackowiak, & Turner, 1996) and signals from white matter and the ventricles were regressed to account for physiological noise. To further account for the fact that measures of rs-FC may be influenced by head motion, each individual's movement during scanning was quantified using framewise displacement (FD) (Power, Barnes, Snyder, Schlaggar, & Petersen, 2013). FD is a compressed single index calculated from derivatives of the six rigid-body realignment parameters. Volumes exceeding a FD of 0.5mm, a commonly used threshold (Power et al., 2012) were eliminated, otherwise known as scrubbing.

1.9.4. Approaches to analysing rs-FC

During the past two decades, a growing body of neuroimaging research has employed rs-FC techniques to determine whether differences exist in rs-FC between individuals with schizophrenia and controls. There are several approaches to exploring rs-FC, and for the purpose of this thesis I will focus on two; whole-brain and seed-based methods.

1.9.4.1. Whole-brain methods

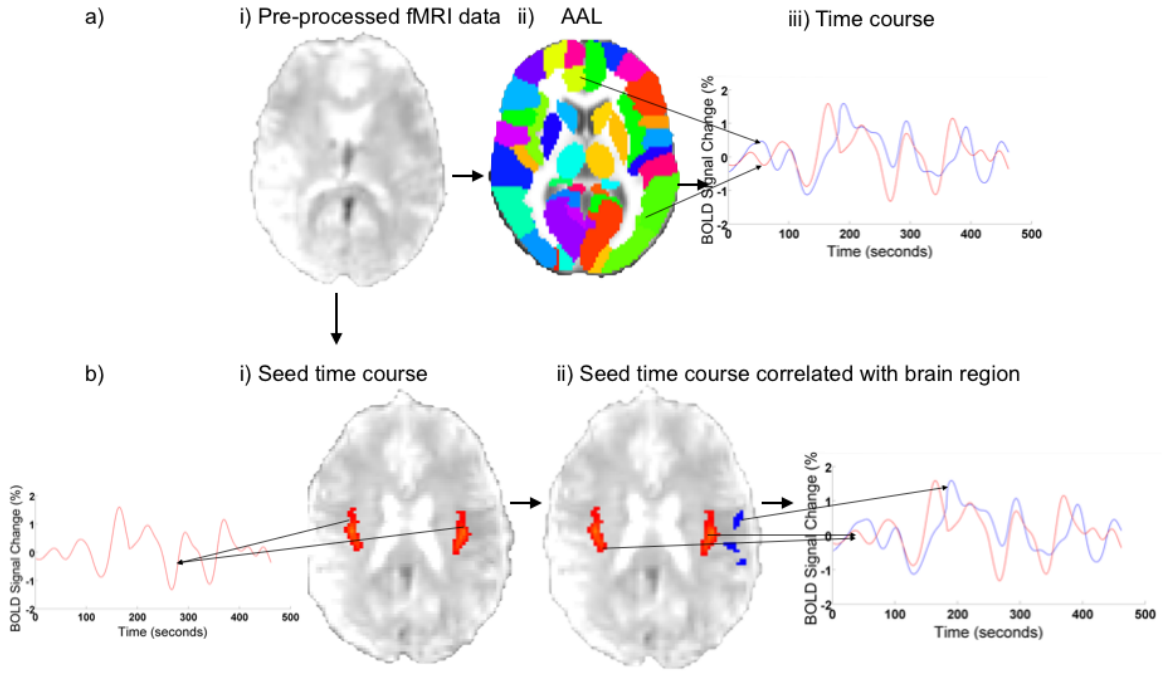
A whole-brain approach to investigating rs-FC, is a data-driven, hypothesis free method that maximises inference and minimizes bias. Whole-brain rs-FC commonly involves anatomically partitioning the brain into distinct regions, often with the application of an

established brain parcellation template that has functionally subdivided the cortex (see Figure 1.2a). The Pearson correlation is then calculated between each possible pair of regions to determine a measure of functional connectivity. The network-based statistic (NBS) (Zalesky, Fornito, & Bullmore, 2010) is then used to control the familywise error rate and identify networks of connections that significantly differ in FC between groups.
This results in an explorative and holistic representation of rs-FC across the entire brain whilst robustly correcting for multiple comparisons.

1.9.4.2. Seed-based methods

Seed-based analyses are hypothesis driven methods for investigating the functional connectivity of a particular brain structure or region. A seed, or region of interest (ROI) is defined, such as the insula, and the mean time series for that region is obtained by averaging the time series of all voxels comprising the insula. Following this, the correlation between the ROI (e.g. insula) and all other grey matter voxels of the brain is computed, resulting in a comprehensive spatial correlation map of the whole brain (Smith et al., 2013), (see Figure 1.2b). Two brain regions showing positive correlations/positive functional connectivity are assumed to be functionally coupled and related, and two regions that are negatively correlated are hypothesized to be functionally anti-correlated and theorised to belong to different functional networks (Fox et al., 2005). Seed-based rs-FC is a well-established reliable approach that provides a clear view of with which regions the chosen ROI is functionally connected to. However, results are confined to the functional connections of that ROI, limiting inferences made to specific parts of the brain and potentially overlooking interesting patterns of connectivity.

Figure 1.2. Whole-brain and seed-based functional connectivity



a) i) Pre-processed fMRI data is parcellated using ii) the AAL template. iii) The time course is then extracted from two anatomical regions and they are correlated.

b) i) The time course is extracted for the specified ROI and is correlated with all other voxels of the brain. ii) A region that has emerged as showing a positive correlation with the specified ROI is denoted in blue.

Note: fMRI = Functional magnetic resonance imaging; AAL = Automated anatomical labelling template; BOLD = Blood oxygen level dependent

1.9.5. Graph theory-based techniques

Recently, in conjunction with measures of functional connectivity, graph theoretical methods have been applied to fMRI data in an attempt to understand the topology and efficiency of brain networks. The advantage of such an approach is that it goes beyond connectivity analyses and facilitates the exploration of complex networks and their properties, such as

efficiency of information transfer on whole brain and regional scales (Rubinov & Sporns, 2010). This approach in conjunction with rs-FC can provide valuable insight into the functional topological architecture in healthy brains, and in disorders such as schizophrenia. A graph-theory based approach models the brain mathematically as a complex network that consists of nodes (brain regions) and edges (functional or structural connections) that exist between nodes (Bassett & Bullmore, 2009; Bullmore & Sporns, 2009; Rubinov & Sporns, 2010; Stam, 2010). Graph measures are computed from the same brain network matrices that are used to compute whole-brain rs-FC. That is, each brain region from the AAL template represents a 'node', and the rs-FC strength between two brain regions (or nodes) represents an 'edge'. A brain networks functional topology can be measured using various computations, and for the purpose of this thesis I will focus on three in particular; global and local efficiency, and small worldness.

1.9.5.1. Global efficiency

Network efficiency broadly measures how efficiently information is exchanged both locally and globally across a network. Global efficiency is related to the shortest pathlength between nodes, that is, the smallest number of edges traversed to get from node A to node B. In turn, the average shortest pathlength of a network is the average of all shortest paths between all possible pairs of nodes. Global efficiency is defined as the inverse of the average shortest pathlength of the network and represents the level of global integration and the speed of parallel information transfer across nodes, where shorter pathlengths between nodes result in higher global efficiency. C*haracteristic path length* is directly related to global efficiency, and is defined as the average shortest path (minimum number of connections traversed) between any two nodes in the network, and reflects how globally integrated the network

topology is (Bullmore & Sporns, 2009).

1.9.5.2. Local efficiency

Relatedly, the local efficiency of a node (i.e node A) is the inverse of the average shortest path that connects all neighbours of node A (nodes that are directly connected to node A) once node A is removed. The local efficiency of the entire network is the mean local efficiency of all nodes comprising that network. Just as global efficiency is a measure of brain-wide integration and communication of information, local efficiency measures how efficient the communication is between direct neighbours of a node, when that node is removed (Latora & Marchiori, 2001). Local efficiency is thus a measure of how resilient a network is to random or targeted attack that results in the loss or removal of a node(s). The *Clustering* coefficient measures how locally clustered the network is, and is directly related to local efficiency (Bullmore & Sporns, 2009).

1.9.5.3. Small-worldness

Human brain networks have a small-world topology, meaning they are both globally and locally efficient. This economic configuration enables the efficient transfer of information with high global integration, high local efficiency and low wiring cost. Small world networks are characterized in terms of two specific properties; high clustering coefficients of nodes and short path lengths between nodes. This configuration supports both locally segregated and globally integrated information-processing. Small-worldness has been characterized in both structural (He, Chen, & Evans, 2007) and functional (Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006) studies in humans. Studies have also shown that small world properties are heritable (Smit, Stam, Posthuma, Boomsma, & de Geus, 2008), related to intelligence (van den Heuvel, Stam, Kahn, & Hulshoff Pol, 2009) and affected by age (Achard &

Bullmore, 2007) and sex (Tian, Wang, Yan, & He, 2011).

1.9.6. References

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CHAPTER 2:

Resting-state functional brain networks in first-episode psychosis: a 12-month follow-up study

Research

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Resting-state functional brain networks in first-episode psychosis: A 12-month follow-up study

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Abstract

Introduction: Schizophrenia is increasingly conceived as a disorder of brain network connectivity and organization. However, reports of network abnormalities during the early illness stage of psychosis are mixed. This study adopted a data-driven whole-brain approach to investigate functional connectivity and network architecture in a first-episode psychosis cohort relative to healthy controls and whether functional network properties changed abnormally over a 12-month period in first-episode psychosis.

Methods: Resting-state functional connectivity was performed at two time points. At baseline, 29 first-episode psychosis individuals and 30 healthy controls were assessed, and at 12 months, 14 first-episode psychosis individuals and 20 healthy controls completed follow-up. Whole-brain resting-state functional connectivity networks were mapped for each individual and analyzed using graph theory to investigate whether network abnormalities associated with first-episode psychosis were evident and whether functional network properties changed abnormally over 12 months relative to controls.

Results: This study found no evidence of abnormal resting-state functional connectivity or topology in first-episode psychosis individuals relative to healthy controls at baseline or at 12-months follow-up. Furthermore, longitudinal changes in network properties over a 12-month period did not significantly differ between first-episode psychosis individuals and healthy control. Network measures did not significantly correlate with symptomatology, duration of illness or antipsychotic medication.

Conclusions: This is the first study to show unaffected resting-state functional connectivity and topology in the early psychosis stage of illness. In light of previous literature, this suggests that a subgroup of first-episode psychosis individuals who have a neurotypical resting-state functional connectivity and topology may exist. Our preliminary longitudinal analyses indicate that there also does not appear to be deterioration in these network properties over a 12-month period. Future research in a larger sample is necessary to confirm our longitudinal findings.

Keywords

Resting state, first episode of psychosis, functional connectivity, graph theory

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Introduction

The disconnection hypothesis of schizophrenia suggests that dysfunctional integration and communication among brain regions is central to illness pathology (Friston and Frith, 1995), a notion that continues to gain support across the psychosis field. Resting-state functional magnetic resonance imaging (rs-fMRI) studies have identified abnormal functional connectivity (rs-FC) at various stages of psychosis. The majority of past research has been conducted in established schizophrenia patient cohorts and, despite somewhat mixed results, the prevailing findings support decreased rs-FC in patients, primarily involving frontal and temporal brain regions (for review, see Yu et al. (2012)). However, fewer studies have examined the nature of rs-FC disturbances during the first-episode illness stage of psychosis.

In individuals experiencing first-episode psychosis (FEP), reports of abnormal rs-FC are divergent, partially due to the widely varied methods and lack of a data-driven approach across research groups (Greicius, 2008). Only four studies have conducted whole-brain analyses of rs-FC networks in FEP and reported widespread FC reductions (Argyelan et al., 2015), reduced inter-hemispheric rs-FC (Guo et al., 2014, 2017), and both increased and decreased rs-FC between various frontal, temporal and subcortical regions in FEP groups relative to controls (Li et al., 2016b). FEP studies that have constrained the investigation of rs-FC to hypothesized region(s) of interest (ROI) vary greatly in the specified ROI and, unsurprisingly, have reported diverse results of both increased rs-FC (Cui et al., 2015; Fornito et al., 2013; Gong et al., 2016; Guo et al., 2015; Li et al., 2016b) and decreased rs-FC (Cui et al., 2015; Fornito et al., 2013; Gong et al., 2016; Guo et al., 2014, 2015; Li et al., 2016b) in FEP participants relative to controls.

In addition to the employment of widely varying methodologies to map rs-FC, it is possible that the heterogeneity of findings at the earlier stages of illness reflect differing stages of progressive structural brain changes on a background of dynamic maturational brain changes (Cropley and Pantelis, 2014; Pantelis et al., 2009). In light of the highly varied rs-FC literature, it appears that the classic approaches of characterizing rs-FC cross-sectionally are not sensitive or specific enough to elucidate network-level abnormalities that might underpin the onset of psychosis. Thus, mapping brain function more extensively and longitudinally presents an important way forward.

In addition to employing a longitudinal design, another way to enhance our understanding of the neurobiological functional differences and changes that are present during the FEP illness phase, and which may help explain the inconsistent rs-FC findings, is by broadening our scope to explore other features of connectivity using graph theory. Recently, in conjunction with measures of FC, graph-theoretical methods have been applied to fMRI data in an attempt to understand the topology and efficiency of brain networks. The advantage of such an approach is that it goes beyond connectivity analyses and facilitates the exploration of complex networks and metrics, such as efficiency of information transfer on whole-brain and regional scales (Rubinov and Sporns, 2010). Previous studies have reported abnormal structural (Fornito et al., 2012; Zalesky et al., 2011) and functional brain network topology in established schizophrenia (Hadley et al., 2016; Lo et al., 2015; Lynall et al., 2010); however, few studies have used graph theory to understand network organization in FEP. While structural network topology has been found to be abnormal in early illness stages (Crossley et al., 2017; Hu et al., 2016; Palaniyappan et al., 2016; Zhang et al., 2015), to our knowledge, no study has examined functional network topology during resting state in FEP longitudinally. Investigating functional brain networks in psychosis using graph-theoretical methods may better elucidate the interplay between network arrangement and communication during the early stages of the disorder and how network properties may change over time.

To bring clarity to the disparate research on rs-FC in FEP we adopted a novel, complementary data-driven approach focusing on FC and well-established graph metrics to characterize both local and global brain network properties in FEP individuals. We mapped resting-state functional brain networks in FEP individuals and healthy controls at baseline and at 12 months follow-up to address the following three aims: (1) to investigate whether rs-FC and topological descriptors of functional brain networks significantly differed between FEP individuals and healthy age- and sex-matched controls; (2) to test whether any longitudinal rs-FC changes in FEP individuals between baseline and 12 month follow-up were more pronounced than expected due to healthy development and (3) to investigate whether any longitudinal changes in rs-FC correlated with measures of clinical characteristics. We hypothesized that FEP participants would show reduced rs-FC and reduced network efficiency relative to controls at both time points and that network properties would change abnormally over a 12-month period.

Methods

Participants

A total of 29 FEP participants (mean age = 19.5 ± 2.3 years, 22 males) were recruited through Orygen Youth Health (OYH) clinical sites in Victoria, Australia. Inclusion criteria for FEP participants consisted of meeting the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., test revision; *DSM-IV-TR*) Axis I diagnosis of a first-episode psychotic disorder with an illness duration of less than 2 years (American Psychiatric Association., 2000). Exclusion criteria

for FEP participants included psychotic symptoms that were possibly attributable to acute intoxication or drug use. In all, 30 healthy control participants (mean age = 22.6 ± 3.2 years, 20 males) were recruited from the general community. Inclusion criteria for healthy controls included psychiatrically, neurologically and medically healthy, as determined by self-report, the research version of the structured clinical interview for *DSM*-IV-TR Axis I disorders (SCID), and the first four subscales of the Comprehensive Assessment of At Risk Mental States (CAARMS; Yung et al., 2005).

General exclusion criteria for all participants included any history of significant head injury, previous or current history of neurological disease, the presence of standard magnetic resonance imaging (MRI) contraindications (e.g. metal implants, claustrophobia, current pregnancy or breastfeeding), premorbid IQ < 70 and a documented history of developmental delay or intellectual disability. Clinical symptoms were assessed using the Brief Psychiatric Rating Scale (BPRS; Andersen et al., 1989), the Scale for the Assessment of Negative Symptoms (SANS; Andreasen, 1989) and the Montgomery–Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg, 1979). In addition, all participants were evaluated using the Social and Occupational Functioning Assessment Scale (SOFAS; Goldman et al., 1992). Due to participants who withdrew or did not complete follow-up assessments, 14 FEP and 20 control participants had 12-month imaging and clinical data available. The study was approved by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069); and all participants provided written informed consent prior to participation.

Imaging data acquisition

Magnetic resonance images were acquired on a Siemens Avanto 3T Magnetom Trio Tim scanner. T1-weighted images were acquired using an optimized Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence. The sagittal imaging parameters were 176 sagittal slices of 1 mm thickness without gap, field of view (FOV) = 250 × 250 mm^2, repetition time (TR) = 1980 ms, echo time (TE) = 4.3 ms, flip angle = 15°, using an acquisition matrix of 256 × 256 resulting in a final reconstructed voxel resolution of 0.98 × 0.98 × 1.0 mm^3. Resting-state fMRI was acquired using T2*-weighted echo-planar imaging (TE = 40 ms; TR = 2.4 s; voxel dimensions = 3.3 × 3.3 × 3; matrix size = 64 × 64). Resting-state fMRI data were acquired for 8 minutes, resulting in 200 volumes. During the acquisition, participants were asked to close their eyes and let their minds wander without going to sleep.

fMRI data preprocessing

Data preprocessing was performed using FSL (FMRIB Software Library, www.fmrib.ox.ac.uk) and SPM8 (www.fil.ion.ucl.ac.uk/spm). For each subject, echo-planar images were slice-time corrected, realigned to the mean functional image to correct for motion, co-registered to their structural T1-weighted scan via rigid-body registration and then spatially normalized by non-linear registration to the Montreal Neurological Institute (MNI) 152 template with 2 mm resolution. Data were spatially smoothed using a Gaussian kernel of full width at half maximum 4 mm and bandpass filtered (0.01–0.1 Hz). Head motion was controlled with the Friston 24-parameter model (Friston et al., 1996), and signals from white matter and the ventricles were regressed to account for physiological noise. The global signal was not regressed due to ongoing controversy surrounding whether this step is warranted when mapping rs-FC (Yang et al., 2014). Given that measures of rs-FC may be influenced by head motion (Power et al., 2012), each individual's movement during scanning was quantified using framewise displacement (FD; Power et al., 2013). FD is a compressed single index calculated from derivatives of the six rigid-body realignment parameters. Volumes exceeding an FD of 0.5 mm, a commonly used threshold (Power et al., 2012), were eliminated, otherwise known as scrubbing.

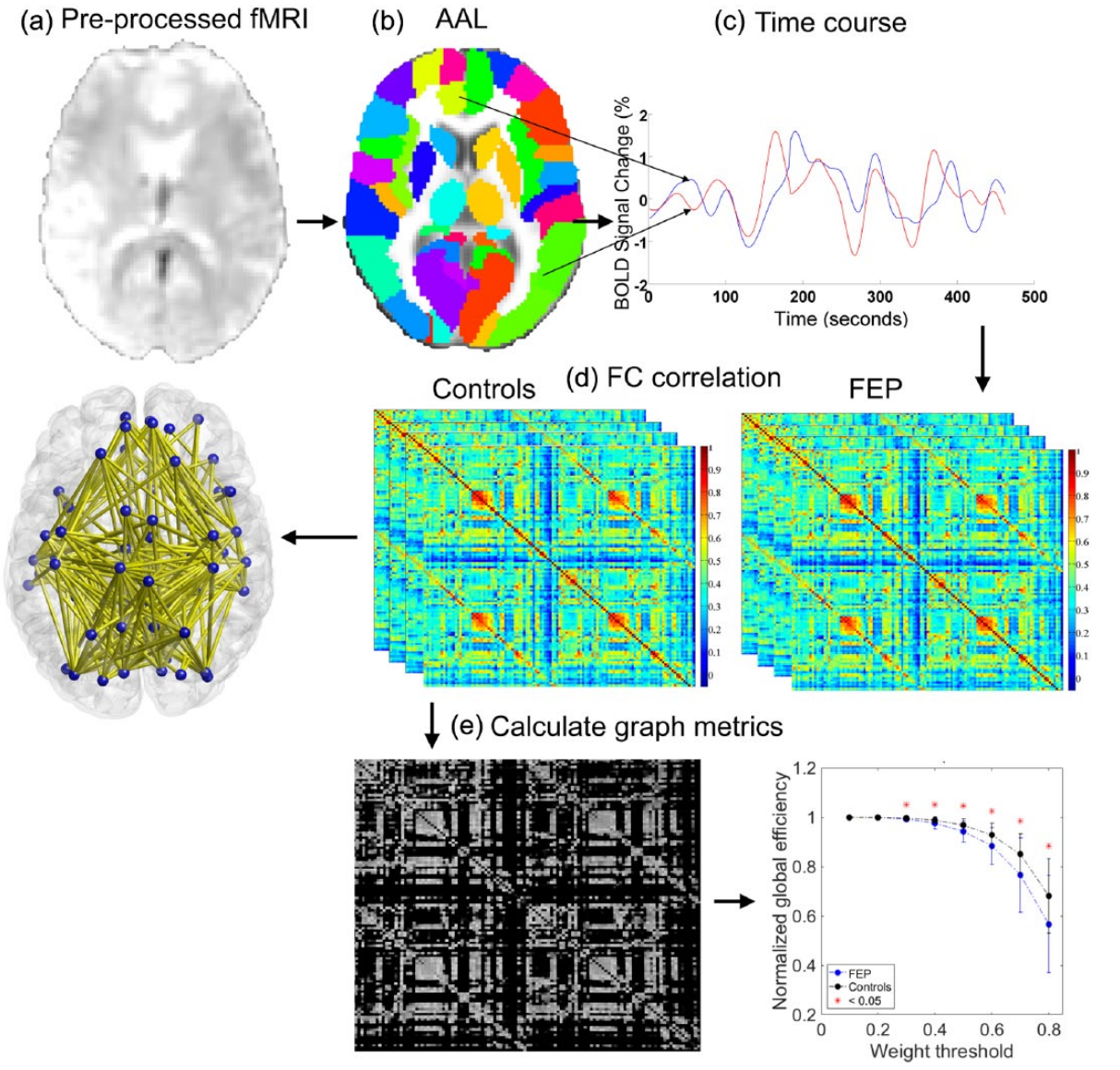
Whole-brain functional network mapping

See Figure 1 for an overview of the methodology used. For each individual, a whole-brain resting-state functional network was mapped using established methods (Fornito et al., 2016). In brief, regionally averaged fMRI signals were determined for the $N = 116$ regions automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). For each individual, regionally averaged fMRI signals were then correlated in a pairwise manner using Pearson correlation, resulting in a $N \times N$ rs-FC matrix. The network-based statistic (NBS; Zalesky et al., 2010) was used to control the family-wise error rate and identify networks of connections that significantly differed in FC between groups.

Graph-theoretical analyses

Seven commonly studied graph-theoretical measures were computed: (1) *Local efficiency* was used to characterize how well the neighbors of a given brain region remain connected after that region and its associated connections are removed from the network (Latora and Marchiori, 2001); (2) *Global efficiency*, a measure of network integration that captures a system's ability to exchange information in a parallel manner, was also investigated (Bullmore and Sporns, 2012); (3) *Characteristic path length* is inversely related to global efficiency and is defined as the average shortest path (minimum number of connections traversed) between any two nodes in the network and reflects how globally integrated the network topology is (Bullmore and Sporns, 2009); (4) The *Clustering coefficient* measures

Figure 1. (a) Pre-processed and registered fMRI data were parcellated using (b) the AAL template. (c) The time course was extracted from each anatomical node. (d) A 116 × 116 connectivity matrix was then generated for each participant quantifying the extent of inter-regional FC between each pair of regions. The NBS was then used to identify the network of connections that significantly differed in FC between patients and controls. (e) Weight thresholding was performed by eliminating all connections below the chosen threshold and binarising the resulting network. Graph metrics including normalized global and local efficiency were then calculated.



FEP: first-episode psychosis; FC: functional connectivity; AAL: automated anatomical labeling system; fMRI: functional magnetic resonance imaging.

how locally clustered the network is and is directly related to local efficiency (Bullmore and Sporns, 2009) and (5) Small-world networks are characterized in terms of two specific properties—high clustering coefficients of nodes and short path lengths between nodes. This configuration supports both locally segregated and globally integrated information processing. *Small worldness* was determined by dividing the normalized clustering coefficient by the normalized path length (Bullmore and Sporns, 2009). A network is said to have small-world topology when small worldness > 1 (Achard et al., 2006). (6) *Degree* of a node is the number of other nodes to which it is directly connected (Bullmore and Sporns, 2009), and the *average degree* and *degree variance* were obtained by taking the mean and standard deviation, respectively, of the degree of all nodes in the network. (7) *Robustness (targeted attack)* involves systematically removing nodes in order of degree, and each time a node is removed from the network, the size of the

largest connected component is calculated (Lynall et al., 2010). All measures (except for degree, degree variance and robustness) were normalized with respect to 100 degree-matched random networks generated with the Maslov–Sneppen rewiring algorithm (Milo et al., 2002). The first six graph measures were computed for graphs that were binarized with respect to a series of connection weight thresholds ranging between 0.1 and 0.6 applied to rs-FC matrices of each individual (Rubinov and Sporns, 2010), and the area under curve (AUC) was used as a summary statistic for each measure (Lynall et al., 2010). Robustness was determined by applying connection weight thresholds ranging between 0.2 and 0.8, and the mean AUC of the graph of largest connected component versus the number of nodes removed at each weight threshold was used as a summary statistic (Achard et al., 2006).

Statistical analyses exploring rs-FC and graph theory measures

We tested the following two hypotheses: (1) whether there was a difference in rs-FC and network topology between FEP individuals and healthy controls at baseline as well as at follow-up and (2) whether there was a difference between the two groups in the 12-month *change* in rs-FC and network topology. The NBS was applied with a primary *T*-statistic threshold of 3 ($p=0.01$), and 10,000 permutations were generated to construct an empirical null distribution for the size of the maximal sub networks that showed group differences in rs-FC between FEP and control groups. Analyses of variance (ANOVAs) were used to test the null hypothesis of equality in the AUC of graph measures between the FEP and control groups at baseline and at follow-up. A repeated measures ANOVA (2×2) was used to investigate whether there was a difference between the two groups in *change* in graph measures over 12 months. The main effect of time and the interaction between time (baseline vs follow-up) and group (FEP vs healthy controls) was tested.

Functional network properties and symptomatology/functioning

Pearson correlation was used to test for an association between rs-FC and graph theory measures with the five BPRS subscales (positive, agitation/mania, negative, depression/anxiety and total score; Ventura et al., 2000) and illness duration in the FEP group and four SANS subscales (anhedonia/avolition, flat affect/alogia, attention and total score), MADRS total score and SOFAS score in both FEP and control groups (groups analyzed separately) at both baseline and at follow-up. A repeated measures *t*-test including symptom change as a covariate of interest was performed in the FEP group to investigate whether any longitudinal change in rs-FC or topology correlated with symptom change over time.

Results

Demographics

Demographic information is shown in Tables 1 and 2. FEP individuals were statistically matched in terms of mean age and gender. No network measure was significantly correlated with age, duration of illness, medication dosage (chlorpromazine equivalent dosage) or IQ ($p>0.05$); therefore, these variables were not included as nuisance confounds. Of the 29 FEP individuals recruited, 14 completed the 12-month follow-up assessment. Relative to FEP individuals who did not participate in the follow-up assessment, participating FEP individuals had significantly lower BPRS total symptom scores at baseline ($t(26)=2.31$, $p=0.029$), and FEP individuals with follow-up data did not significantly differ from those without follow-up data at baseline in IQ, medication dosage, SOFAS score, MADRS total score or SANS total score. Of the 29 FEP participants, 22 (missing data=1) at baseline and 10 of the 14 FEP participants (missing data=2) at 12-month follow-up were on antipsychotic medication at the time of assessment. In the FEP group, diagnosis did not change from baseline at 12-month follow-up. There were no significant differences in functional network properties and clinical characteristics between participants who were and were not prescribed antipsychotic medication at baseline or at 12-month follow-up.

Whole-brain FC

Analyses confirmed the null hypothesis of equality in rs-FC between FEP participants and healthy controls for all pairs of brain regions ($p>0.05$; see Tables 3 and 4 and Figure 2(a)). This result was found at baseline and follow-up. To aid in the visualization of results, further analyses were undertaken in which the 116 AAL regions were grouped into six brain lobes according to their anatomical location: frontal, parietal, temporal, occipital, subcortical and posterior fossa. Figure 2(b) shows an effect-size map of rs-FC between-group differences (Cohen's *d*) at baseline and at follow-up. Although there were no significant differences in lobe-to-lobe rs-FC at baseline or at follow-up, frontal, temporal and occipital lobes show the strongest effect sizes at baseline, and posterior fossa, temporal and occipital lobes showed the strongest effects at 12-month follow-up.

Network topology

Analyses confirmed the null hypothesis of equality between FEP participants and healthy controls in the seven graph measures tested at baseline and at 12-month follow-up ($p>0.05$; see Table 3). In addition, we re-examined the FEP group dividing them based on a diagnosis of schizoaffective disorder vs a diagnosis of schizophrenia. We did not find any significant differences in any functional network measure between the two groups.

Table 1. Demographic and clinical information at baseline.

	FEP patients ($n = 29$), mean (SD)	Controls ($n = 30$), mean (SD)	Between-group differences
Gender (male/female)	22/7	20/10	χ^2 (1, $N = 58$) = 1.03, $p = 0.39$
Age (year)	21.3 (2.0)	22.6 (3.2)	$t(58) = 1.83$, $p = 0.07$
Illness duration (year)	1.4 (0.9)	–	–
Age of illness onset (year)	19.5 (2.3)	–	–
IQ	95.3 (17.3)	119.9 (8.8)	$t(56) = 6.69$, $p < 0.0001$**
Education (years)	12.52 (2.5)	15.3 (2.0)	$t(55) = 4.69$, $p < 0.0001$**
SOFAS	56.1 (10.1)	85.8 (6.9)	$t(58) = 13.17$, $p < 0.0001$**
Chlorpromazine equivalent dosage (mg/day)	360.9 (366.0)	–	–
BPRS scores			
Positive symptoms	11.4(4.4)	–	–
Agitation/mania	8.2 (3.5)	–	–
Negative symptoms	4.7 (2.1)	–	–
Depression/anxiety	8.9 (4.9)	–	–
Total	37.6 (9.4)	–	–
SANS scores			
Anhedonia/avolition	11.5 (6.6)	1.8 (3.0)	$t(58) = -7.4$, $p < 0.0001$**
Flat affect/alogia	8.2 (8.1)	2.4 (4.6)	$t(58) = -3.4$, $p < 0.0001$**
Attention	5.0 (3.5)	1.3 (1.8)	$t(58) = -5.3$, $p = 0.012$
Total	24.7 (13.3)	5.5 (6.4)	$t(58) = -7.1$, $p < 0.0001$**
MADRS score (total)	13 (9.4)	3.2 (2.7)	$t(58) = -5.5$, $p < 0.0001$**

FEP: first-episode psychosis; IQ: intelligence quotient; SOFAS: social and occupational functioning assessment scale; BPRS: Brief Psychiatric Rating Scale; SANS: Scale for the Assessment of Negative Symptoms; MADRS: Montgomery Åsberg Depression Rating Scale.
Note. **Significant $p < 0.0001$

Longitudinal changes

We found no evidence of an interaction effect between time and group on rs-FC and graph measures over a 12-month follow-up period (see Table 4). There was also no evidence of a main effect of time on rs-FC and graph measures over the 12-month follow-up period in either the controls or the FEP participants (see Table 4).

Relationships with symptomatology/ functioning

There were no significant associations between symptom severity, social functioning or illness duration and measures of rs-FC strength and network topology at baseline or at 12-month follow-up. Symptom scores did not significantly change over the 12-month follow-up period in FEP participants, and there was no significant association between symptoms change and change in network measures over time.

Discussion

This study found no significant difference in rs-FC and network topology in FEP participants relative to healthy controls, and no evidence of change over a 12-month period. We also found no significant associations between network measures, social functioning and clinical characteristics. With an abundance of evidence reporting rs-FC abnormalities during the early illness stage of psychosis, the present findings provide novel insights regarding rs-FC integrity and topology in FEP both cross-sectionally and longitudinally.

Rs-FC in FEP is comparable to healthy individuals

To our knowledge, this is the first study to report no significant difference in rs-FC in FEP individuals relative to healthy controls. Although the current results do not support the prevailing findings of aberrant rs-FC, they add

Table 2. Demographic and clinical information at 12-month follow-up.

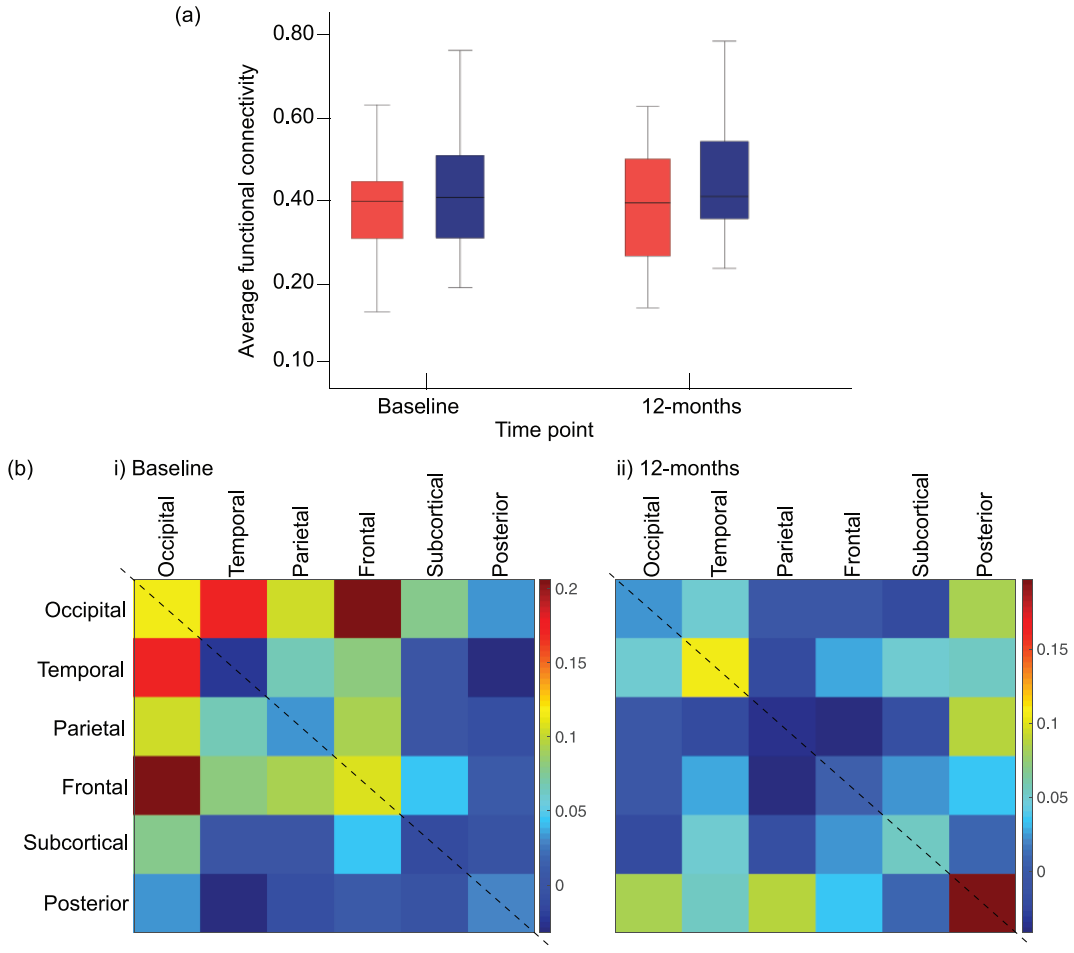
	FEP patients ($n = 14$)	Controls ($n = 20$)	Between-group differences
Gender (male/female)	12/2	13/7	χ^2 (1, $N = 34$) = 1.33, $p = 0.23$
Illness duration (year)	2.5 (1.5)	–	–
SOFAS	58.3 (13.3)	81.6 (6.7)	$t(32) = 6.4$, $p = 0.000$**
Chlorpromazine equivalent dosage (mg/day)	617.4 (151.6)	–	–
BPRS scores			
Positive symptoms	9.9 (3.8)	–	–
Agitation/mania	7.8 (2.2)	–	–
Negative symptoms	6.0 (2.3)	–	–
Depression/anxiety	8.0 (3.5)	–	–
Total	31.7 (4.0)	–	–
SANS scores			
Anhedonia/avolition	10.4 (9.5)	2.6 (3.0)	$t(32) = -3.5$, $p = 0.001$
Flat affect/alogia	5.3 (5.4)	3.0 (7.0)	$t(32) = -1.0$, $p = 0.31$
Attention	5.4 (2.6)	1.7 (2.3)	$t(32) = -4.3$, $p = 0.000$**
Total	21.1 (11.1)	7.2 (8.7)	$t(32) = -4.1$, $p = 0.000$**
MADRS score (total)	10.3 (6.9)	4.4 (4.9)	$t(31) = -2.9$, $p = 0.007$

FEP: first-episode psychosis; IQ: intelligence quotient; SOFAS: social and occupational functioning assessment scale; BPRS: Brief Psychiatric Rating Scale; SANS: Scale for the Assessment of Negative Symptoms; MADRS: Montgomery Åsberg Depression Rating Scale.
Note. **Significant $p < 0.0001$

valuable insight to the existing pool of mixed literature. Our study adopted a data-driven approach to maximize inference, minimize bias and add to the handful of previous studies that used similar brain-wide analyses. Previous whole-brain rs-FC studies have reported both increased (Argyelan et al., 2015; Li et al., 2016b) and decreased (Guo et al., 2014, 2017; Li et al., 2016a) rs-FC in FEP cohorts relative to controls. Despite these studies adopting a similar data-driven approach, results are inconsistent, both in the direction of abnormal rs-FC reported and the specific brain regions implicated. These mixed findings in addition to the current finding of no impairment in FEP suggest that many factors are likely playing a role in mediating and/or moderating rs-FC during the early illness phase. For example, illness duration can vary across studies from months (Li et al., 2016b) to multiple years (Argyelan et al., 2015; Guo et al., 2017); consequently, moderating factors such as duration of medication use and illness are highly varied across cohorts, both of which have been shown to have an effect on rs-FC in FEP (Sarpal et al., 2015, 2017). Interestingly, this study found no significant relationship between network measures and IQ, education, medication use or illness duration both cross-sectionally and over time. Relatedly, our FEP sample had lower total symptom scores relative to previous whole-brain studies (Guo et al., 2014; Li et al., 2016b), placing them in a mild to moderately ill bracket (Leucht et al., 2005). This may be indicative of effective response to antipsychotic medication and/or psychosocial therapy, and our finding of no rs-FC impairment may reflect this. Results from longitudinal rs-FC studies in drug naïve FEP participants following their first antipsychotic medication administration indicate a progressive increase in rs-FC strength that correlated with symptom improvement (Sarpal et al., 2015). It is therefore possible that prior to the commencement of this study, effective antipsychotic treatment normalized any rs-FC or topological abnormalities that were present in this FEP cohort to levels observed in controls. If this is the case, it is an extremely encouraging notion that early intervention and/or effective pharmacological treatment can either reverse neural abnormalities (if they were ever present) or prolong/prevent their emergence over the observed 12-month period.

This, however, is speculative, and there is no way of testing this within the design of this study. Although our FEP cohort did not have the shortest illness duration compared to other studies (mean 1.5 years), they were all recruited from a specialized early intervention service, which likely provided swift and targeted treatment that may have minimized the duration of untreated psychosis (DUP). DUP has been associated with a number of outcomes, including increased symptom severity, poor prognosis (Penttila et al., 2014) and reduced rs-FC between regions of the striatum (Sarpal et al., 2017). Unfortunately, this study did not have information pertaining to DUP, and thus, any

Figure 2. (a) Group differences in mean rs-FC across the whole brain between FEP (red) and control (blue) groups at baseline and 12-month follow-up. (b) Matrices showing the effect size of rs-FC between-group differences at (i) baseline and (ii) at 12-month follow-up for each brain lobe. Effect sizes quantified with Cohen's *d*.



inferences made are speculative. However, it is possible that early and effective treatment from a specialized psychosis intervention service played a role in these individuals continuing to develop along a neurodevelopmental trajectory similar to that observed in controls.

Functional network topology in FEP is comparable to healthy controls

Contrary to our hypothesis, we found no evidence that FEP networks differed topologically relative to controls in any of the graph-theoretical measures tested. This finding is in line with results by Fornito et al. (2011), who investigated functional topology during a cognitive control task and found intact functional network architecture in FEP individuals relative to controls (Fornito et al., 2011). Taken together, these results suggest that the functional efficiency of brain-wide information exchange, and local integration and communication of information at the nodal level (Fornito et al., 2012), is not impaired in FEP relative to controls. Given that previous studies have shown abnormal structural topology in FEP (Crossley et al., 2017; Hu et al., 2016; Palaniyappan et al., 2016; Zhang et al., 2015), future research should explore white matter connectivity in addition to structural network topology in this and other FEP samples to further determine whether structural network architecture is also intact. Again, our finding of no functional topological impairment in FEP may reflect their relatively good clinical state and prognosis and/or the efficacy of their treatment.

Change in rs-FC over time in FEP was comparable to controls

There is an ongoing question surrounding whether psychosis is a progressive brain disorder involving trait neural abnormalities that are present at illness onset and progressively worsen as the illness becomes more established

Table 3. Functional connectivity and network topology metrics at baseline and at 12-month follow-up.

	Baseline			12-month follow-up		
	FEP ($n=29$) (mean ± SD)	Controls ($n=30$) (mean ± SD)	Between-group differences	FEP ($n=14$) (mean ± SD)	Controls ($n=20$) (mean ± SD)	Between-group differences
Connectivity strength	0.41 (0.12)	0.44 (0.14)	$p=0.47$	0.40 (0.14)	0.42 (0.13)	$p=0.72$
Global efficiency	0.66 (0.025)	0.66 (0.025)	$p=0.70$	0.66 (0.027)	0.66 (0.024)	$p=0.69$
Local efficiency	0.94 (0.26)	0.86 (0.23)	$p=0.22$	0.91 (0.24)	0.85 (0.170)	$p=0.44$
Characteristic path length	0.30 (0.0020)	0.303 (0.0037)	$p=0.18$	0.25 (0.002)	0.25 (0.005)	$p=0.13$
Clustering	1.072 (0.32)	0.97 (0.31)	$p=0.22$	1.05 (0.33)	0.96 (0.23)	$p=0.38$
Degree	38.70 (10.89)	41.88 (14.40)	$p=0.30$	38.86(11.84)	40.02 (11.89)	$p=0.78$
Degree variance	11.99 (2.24)	12.76 (2.88)	$p=0.26$	5.54 (1.00)	5.81 (1.20)	$p=0.51$
Robustness (targeted attack)	5.45e+03 (422.98)	5.603e+03 (426.47)	$p=0.14$	5.42e+03 (475.18)	5.59e+03 (439.39)	$p=0.29$
Small worldness	3.55 (1.051)	3.24 (1.10)	$p=0.35$	3.97 (1.16)	3.86 (0.90)	$p=0.76$

FEP: first-episode psychosis; SD: standard deviation.

Table 4. Functional connectivity and network topology metrics change over 12 months.

	Group × time interaction	Effect size[a] (group × time)	FEP × time	Controls × time
Connectivity strength	$F=0.014$, $p=0.91$	0.000	$t=-0.6$, $p=0.56$	$t=-1.09$, $p=0.29$
Global efficiency	$F=0.001$, $p=0.98$	0.000	$t=-0.46$, $p=0.65$	$t=-0.66$, $p=0.52$
Local efficiency	$F=0.020$, $p=0.89$	0.001	$t=-0.26$, $p=0.80$	$t=-0.53$, $p=0.60$
Characteristic path length	$F=1.48$, $p=0.237$	0.066	$t=-0.39$, $p=0.62$	$t=-0.48$, $p=0.51$
Clustering	$F=0.007$, $p=0.94$	0.000	$t=-0.22$, $p=0.83$	$t=-0.39$, $p=0.70$
Degree	$F=0.081$, $p=0.78$	0.003	$t=-0.35$, $p=0.73$	$t=-1.04$, $p=0.31$
Degree variance	$F=-0.34$, $p=0.56$	0.011	$t=-0.58$, $p=0.45$	$t=-0.40$, $p=0.49$
Robustness (targeted attack)	$F=0.021$, $p=0.89$	0.055	$t=0.37$, $p=0.72$	$t=0.57$, $p=0.57$
Small worldness	$F=0.019$, $p=0.89$	0.019	$t=0.63$, $p=0.56$	$t=1.51$, $p=0.15$

FEP: first-episode psychosis.
[a]The effect-size partial eta-squared is reported.

(Pantelis et al., 2009). To further explore this theory, we investigated whether rs-FC and topology changed abnormally in FEP individuals relative to controls over a 12-month follow-up period, and contrary to our hypothesis, we found no evidence of aberrant rs-FC change relative to that of normal development in controls. It is worth mentioning that our FEP group did meet criteria for FEP at baseline. However, there would be increased diagnostic stability in terms of type of psychosis at 12-month follow-up, given the average illness duration is now >2 years. Thus, recent-onset psychosis may be considered a better categorization for this sample at follow-up. Research has shown that roughly 55–60% of individuals who experience FEP go on to have a long-term functional disability (Menezes, Arenovich, & Zipursky, 2006). The notion that 40–45% of FEP individuals do not, questions the theory that early progressive neuropathological deficits are expected to be present during the first episode of psychosis.

FEP individuals are at a point along the psychosis trajectory when schizophrenia is not a stable diagnosis nor is it an inevitable outcome; thus, it is perhaps unsurprising that we found little evidence of a stable neural abnormality associated with the disorder within a 1 year period. Given the clinical heterogeneity of early-onset psychosis, it is possible that different pathological mechanisms are operating at different stages of the illness that this study did not encompass (Bartholomeusz et al., 2017; Pantelis et al., 2005). Furthermore, at baseline, FEP participants who went on to complete a follow-up assessment had significantly lower BPRS total symptoms scores relative to FEP participants that withdrew from the study. It is therefore possible that our follow-up early-onset psychosis cohort are a subgroup of individuals who experienced less severe symptomatology and in turn are more neurobiologically similar to healthy controls and subsequently followed a similar neurodevelopmental trajectory over the 12-month period. Longitudinal designs with longer follow-up periods and larger samples are needed.

Limitations

The stringent brain-wide corrections for multiple comparisons provided excellent control for false-positive results; however, it was consequently more difficult to avoid false negatives (Radua et al., 2012). In addition, the high attrition rate meant we were unable to assess all FEP participants at both time points, resulting in a relatively small follow-up cohort and in turn, this limits the conclusions and inferences that can be made regarding longitudinal changes in rs-FC. We therefore cannot exclude the possibility that we were unable to detect some group differences because of limited statistical power in the longitudinal analyses.

Furthermore, our approach to investigating neural abnormalities in FEP, which largely focused on FC and network efficiency, did not identify potential brain network alterations in this sample, suggesting that clinical presentation may not be driven by aberrant connectivity at this illness stage. However, this was not directly examined in this study, and future studies should consider investigating connectivity (including white matter connectivity), gray matter volumetric alterations and multi-modal approaches and how this correlates with symptomatology and functioning in the early illness stages.

Conclusion

The pathophysiology of schizophrenia is heterogeneous and has been demonstrated to involve multiple brain regions and systems. The early stage of the disorder is most likely a time when neural correlates and mechanisms of psychosis are most varied and difficult to characterize, and the highly mixed literature speaks to this. Early identification and intervention are increasingly viewed as a critical process in order to alter the trajectory of psychosis, and as a field, there is still much to be learned about the neurobiology of this complex and sometimes impermanent illness stage. From a functional perspective, our null findings are encouraging and suggest a more optimistic perception of the illness. It is important for the schizophrenia field to be informed that our results are indicative of a group of FEP individuals who show no observed abnormalities in rs-FC and topology and no evidence of deterioration in the network properties measured over a 12-month period. With this said, a larger sample with a longitudinal design is needed to confirm whether a subgroup of FEP patients who show no rs-FC abnormalities at baseline do display a neurotypical trajectory over time. While our 12-month data might be an indication, it is difficult to make conclusions and inferences with limited statistical power. Further to this, this study does not have any long-term follow-up data to confirm the prognosis of these FEP participants, as some may have essentially recovered, while others may have gone go on to develop a more chronic stable psychotic illness beyond the 12-month follow-up. Therefore, longer follow-up periods are needed to identify the underlying protective mechanisms driving unaffected rs-FC and topology at baseline in early psychosis cohorts.

Given the field is moving toward personalized medicine, the logical next step is to identify under what specific circumstances do a comparatively healthy FC and topological network occur in early-stage psychosis and what neuroprotective factors may be contributing to this neurotypical picture.

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CHAPTER 3:

Functional brain networks in treatment-resistant schizophrenia

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Functional brain networks in treatment-resistant schizophrenia

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ABSTRACT

Introduction: Up to 20% of individuals with schizophrenia show minimal or no response to medication and are considered to have 'treatment-resistant' schizophrenia (TRS). Unlike early and established schizophrenia, few studies have investigated resting-state functional connectivity (rs-FC) in TRS. Here, we test for disruptions in FC and altered efficiency of functional brain networks in a well-characterized cohort of TRS patients.
Methods: Resting-state functional magnetic resonance imaging was used to investigate functional brain networks in 42 TRS participants prescribed clozapine (30 males, mean age = 41.3(10)) and 42 healthy controls (24 males, mean age = 38.4(10)). Graph analysis was used to characterize between-group differences in local and global efficiency of functional brain network organization as well as the strength of FC.
Results: Global brain FC was reduced in TRS patients ($p = 0.0001$). Relative to controls, 3.4% of all functional connections showed reduced strength in TRS ($p < 0.001$), predominantly involving fronto-temporal, fronto-occipital and temporo-occipital connections. Global efficiency was reduced in TRS ($p = 0.0015$), whereas local efficiency was increased ($p = 0.0042$).
Conclusions: TRS is associated with widespread reductions in rs-FC and altered network topology. Increased local functional network efficiency coupled with decreased global efficiency suggests that hub-to-hub connections are preferentially affected in TRS. These findings further our understanding of the neurobiological impairments in TRS.

1. Introduction

Schizophrenia is a severe and chronically debilitating psychiatric disorder, with a two to three-fold increase in early mortality compared with the general population (Saha et al., 2007). Although use of first and second generation antipsychotics has significantly improved treatment response and quality of life for many individuals with schizophrenia, symptoms persist for up to one third of affected individuals, despite trialing various types of antipsychotic medications. This population has been termed "treatment resistant" (i.e., TRS). Currently clozapine is the only evidence-based atypical antipsychotic that has been found effective in ameliorating psychotic symptoms in TRS (Asenjo Lobos et al., 2010). However, clozapine is effective in only a fraction of patients, as up to 70% of TRS individuals do not respond (Papetti et al., 2007). Consequently, TRS is one of the greatest therapeutic challenges, with patients often suffering a more severe and chronic form of the disorder than those who respond to antipsychotic treatment (Bolonna and Kerwin, 2005). Many clinicians have posited that TRS may in fact be more accurately understood as a distinct subtype of schizophrenia, as opposed to being a chronic illness phase (Farooq et al., 2013; Lee et al., 2015). This notion has been supported by recent findings of differences in dopamine concentrations in the limbic and associative striatal subdivisions and glutamate levels in the anterior cingulate cortex between treatment-responsive schizophrenia and TRS groups (Demjaha et al., 2014; Demjaha et al., 2012). The relation between striatal dopamine and disrupted functional connectivity remains unclear in schizophrenia,

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however elevated dopamine levels may worsen the signal-to-noise ratio of spontaneous brain activity in the striatum, leading to a reduction in functional connectivity between striatum and frontal regions (Sorg et al., 2013). Despite the clinical relevance of TRS, few neuroimaging studies have focused on this population.

The theory of 'dysconnectivity' between spatially separated brain systems is one of the most prominent and widely researched hypotheses in schizophrenia (Friston and Frith, 1995; Zalesky et al., 2011; Zalesky et al., 2015). Findings however are inconsistent, with reports of both increased resting-state functional connectivity (rs-FC) (Jafri et al., 2008; Lui et al., 2010; Sorg et al., 2013; Whitfield-Gabrieli et al., 2009) and decreased rs-FC (Bluhm et al., 2007; Bluhm et al., 2009; Camchong et al., 2011; Gavrilescu et al., 2010; Hoptman et al., 2010; Liang et al., 2006; Meda et al., 2012; Ongur et al., 2010; Rotarska-Jagiela et al., 2010; Vercammen et al., 2010; Zhou et al., 2007; Zhou et al., 2008). Few studies have used functional imaging to investigate rs-FC in individuals with TRS. Using independent component analysis, one study found that TRS individuals with auditory-verbal hallucinations (AVH) showed reduced rs-FC between the left temporo-parietal junction and right Broca's area and anterior cingulate cortex (Vercammen et al., 2010). A later study also investigated AVH in TRS and found an increase in connectivity between bilateral temporal regions and a decrease in connectivity within the cingulate cortex (Wolf et al., 2011). These studies however, had relatively small samples ($n = 27$, $n = 10$) and explored connectivity predominantly in the context of AVH (Vercammen et al., 2010; Wolf et al., 2011). The most recent study by White et al. (2016) found reduced FC between the ventral striatum and substantia nigra in TRS compared with non-TRS patients, indicating there may be fundamental differences in network properties (reduced FC) between treatment-responsive and TRS patients.

More recently, in conjunction with measures of FC strength, graph theoretical methods have been applied to functional magnetic resonance imaging (fMRI) data in an attempt to understand the topology of brain networks. Two such measures that address the question of functional network organization are global and local efficiency. The efficiency of a brain network is inversely related to the number of intermediate regions that must be traversed for a pair of brain regions to communicate with each other. A directly connected pair of regions can communicate most efficiently since they do not utilize any intermediate regions. However, many pairs of brain regions are not directly connected, and thus communication between such regions is via a path that traverses one or more intermediate regions. The greater the number of intermediate regions traversed, the less efficient communication becomes, due to increasing energy requirements and potential signal dispersion (Bullmore and Sporns, 2012; Fornito et al., 2016). A reduction in brain network efficiency in patients may indicate a bias in the trade-off between metabolic costs and topology (Rubinov and Sporns, 2010; Wang et al., 2010).

Here, we characterized the connectivity and efficiency of whole-brain functional networks inferred from rs-fMRI in a group of individuals with TRS, compared to healthy controls. We also investigated whether a relationship between network connectivity and topology and symptomatology/functioning is evident. In light of previous research and the chronicity of the present sample, we hypothesize that the TRS group will show widespread reduced rs-FC, predominantly between frontal-temporal regions and topological abnormalities in the form of reduced global efficiency compared with controls. We also hypothesize that these abnormalities will correlate with symptom severity and functioning in the TRS group.

2. Methods

2.1. Participants

Forty-two treatment resistant schizophrenia (TRS) individuals (mean age 41.3 ± 10.0, 30 males) were recruited from inpatient and outpatient clinics in Melbourne, Australia. TRS was defined as at least two unsuccessful trials of two or more different antipsychotic types and currently taking clozapine (Kane et al., 1988; Suzuki et al., 2012).

Inclusion criteria for the TRS group were a diagnosis of schizophrenia, currently prescribed and taking clozapine and aged 18–65 years. Forty-two healthy control participants (mean age 38.4 ± 10.4, 24 males) with similar socio-economic backgrounds were recruited from the general community. All participants were administered the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) to confirm diagnosis of schizophrenia and to rule out current or past psychopathology in healthy controls. Clinical symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), and all participants were evaluated using the Global Assessment of Functioning (GAF) (Hall and Parks, 1995) and the Social and Occupational Functioning Assessment Scale (SOFAS) (Goldman et al., 1992).

The study was approved by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069); and all participants provided written informed consent prior to participation.

2.2. Imaging data acquisition

Magnetic resonance images were acquired on a Siemens Avanto 3T Magnetom TIM Trio scanner. T1-weighted images were acquired using an optimized Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence. The sequence parameters were: 176 sagittal slices of 1 mm thickness without gap, field of view (FOV) = 250×250 mm^2, repetition time (TR) = 1980 ms, echo time (TE) = 4.3 ms, flip angle = 15°, using an acquisition matrix of 256×256 resulting in a final reconstructed voxel resolution of $0.98 \times 0.98 \times 1.0$ mm^3. Resting-state fMRI was acquired using a T2*-weighted echo-planar imaging sequence (TE = 40 ms; TR = 2.4 s; voxel dimensions = $3.3 \times 3.3 \times 3$; matrix size = 64×64). Data was acquired for 8 min, resulting in 200 volumes.

2.3. fMRI data preprocessing

Data was preprocessed using FSL (FMRIB Software Library, www.fmrib.ox.ac.uk) and SPM8 (http://www.fil.ion.ucl.ac.uk/spm). For each subject, echo-planar images were slice-time corrected, realigned to the mean functional image to correct for motion, and co-registered to the structural T1-weighted scan via rigid-body registration then spatially normalized by non-linear registration to the Montreal Neurological Institute (MNI) 152 template with 2 mm resolution. Data was spatially smoothed using a Gaussian kernel of full width at half maximum 4 mm, and bandpass filtered (0.01–0.1 Hz).

Head motion was controlled with the Friston 24-parameter model (Friston et al., 1996) and signals from white matter and the ventricles were regressed to account for physiological noise. Given measures of FC may be influenced by head motion (Power et al., 2012), each participant's movement during scanning was quantified by Framewise Displacement (FD) (Power et al., 2013). FD is a compressed single index calculated from derivatives of the six rigid-body realignment parameters. Volumes exceeding a FD of >0.5 mm, a commonly used threshold (Power et al., 2012) were eliminated, otherwise known as "scrubbing".

2.4. Whole-brain connectivity analysis

An overview of the methodology is shown in Fig. 1. A FC matrix was generated for each subject by anatomically parcellating the registered fMRI volumes into 116 nodes using the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). For each of the 116 regions, the mean time-series was calculated by averaging the fMRI time series across all voxels. The Pearson correlation coefficient was then calculated between each of the distinct regional pairs to determine a measure of FC. The network-based statistic (NBS) (Zalesky et al., 2010) was used

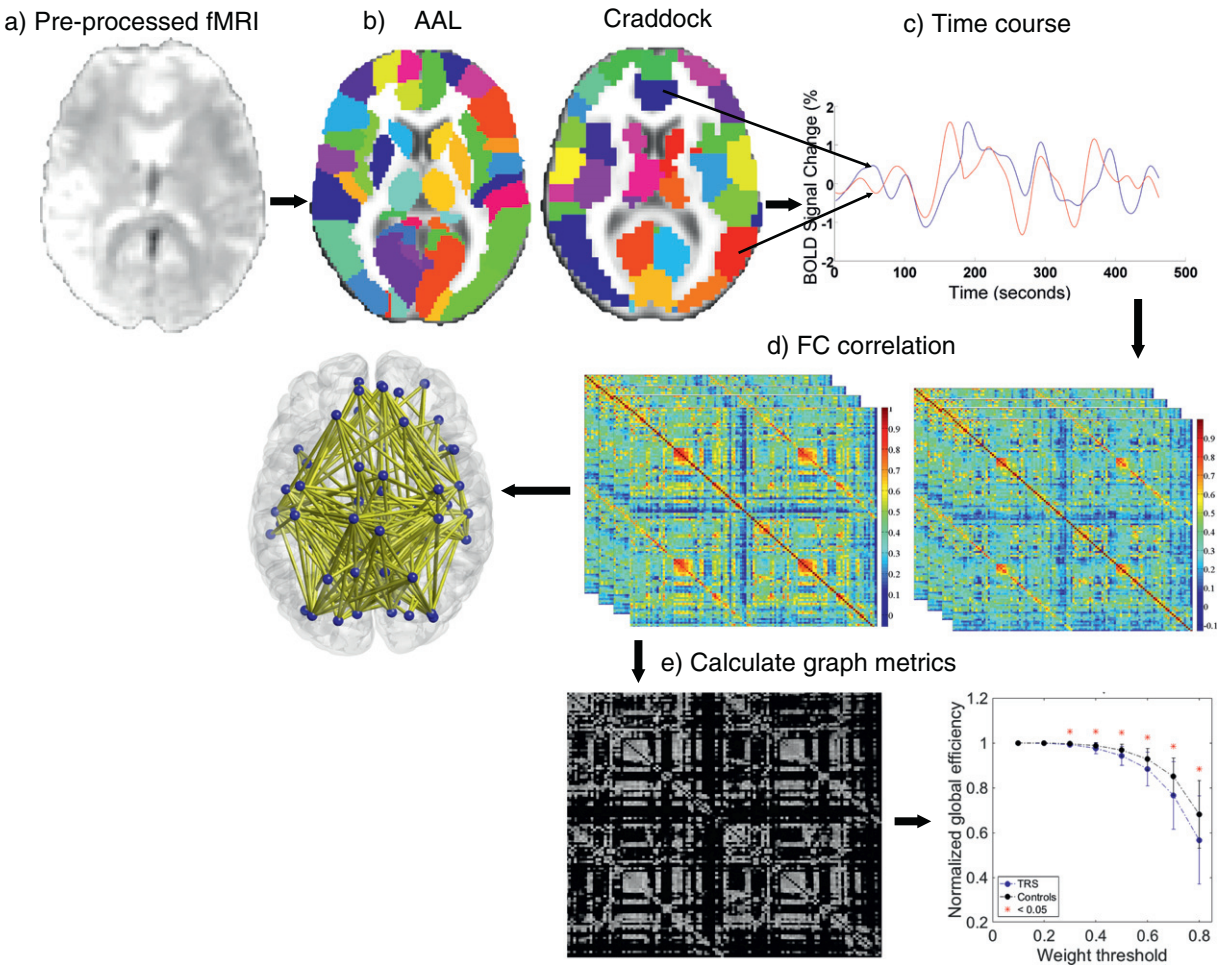

Fig. 1. Methodology overview. a) Pre-processed and registered fMRI data was parcellated using b) the automated anatomical labeling system (AAL) (findings were verified using the Craddock template). c) The time course was extracted from each anatomical node. d) A 116 × 116 connectivity matrix was then generated for each participant quantifying the extent of inter-regional functional connectivity (FC) between each pair of regions. The NBS was then used to identify the network of connections that significantly differed between groups. e) Weight thresholding was performed by eliminating all connections below the chosen threshold and binarising the resulting network.

to identify networks of connections that significantly differed in FC between groups while correcting for multiple comparisons. A conservative primary *t*-statistic threshold of 4 was used to minimize the rate of individual connections for which the null hypothesis was falsely rejected. It is important to remark that control of the family-wise error rate is guaranteed with the NBS irrespective of the primary threshold choice. The use of a conservative primary threshold improves specificity at the level of individual connections. Our findings were replicated with a primary *t*-statistic threshold of 3, albeit a more extensive network of disruptions was identified. To assess the reproducibility of results to alternative atlases, the above analysis was repeated with the Craddock atlas (see Supplementary material Fig. 1).

To assist the interpretation of our findings, we further divided the 116 regions into six brain lobes according to their anatomical location: frontal, parietal, temporal, occipital, subcortical and posterior fossa. We then counted the number of reduced connections in the TRS group between each pair of lobes, or between pairs of regions residing in the same lobe.

2.5. Network efficiency

Global and local network efficiency were computed and compared between the TRS and control groups. Each subject's functional network was transformed into a binary graph by eliminating connections with FC below a fixed threshold, otherwise known as weight-based thresholding (Fornito et al., 2016). A range of FC thresholds between 0.1 and 0.8 were considered. Local and global efficiency were computed for each threshold and normalized with respect to 100 degree-matched random networks generated with the Maslov-Sneppen rewiring algorithm (Milo et al., 2002). Normalization was performed to control for potential differences in connection density between individuals. These normalized measures of efficiency were then compared between TRS and controls at each threshold and the area-under-curve (AUC) was used to provide a summary measure.

2.6. Functional connectivity and symptomatology/functioning

Pearson correlation was used to test for an association between FC and network efficiency and the five PANSS subscales (positive, negative, excitement, disorganized, depressed) and PANSS total score (Wallwork et al., 2012) in the TRS group and GAF and SOFAS scores in the TRS and control groups (groups analyzed separately). For this analysis, each subject was assigned a FC value representing the average FC over all connections associated with a significant between-group difference and an AUC value for both global and local network efficiency.

3. Results

Demographic information is shown in Table 1.

3.1. Whole-brain functional connectivity analyses

Whole-brain connectivity analyses from the NBS revealed reduced FC in the TRS group compared with controls between multiple pairs of regions (228; 3.4%; $p < 0.001$), see Fig. 2. TRS individuals did not exhibit any regional pairs showing increased FC compared with controls. The NBS guarantees weak control of the family-wise error rate, which means we can only make inferences of statistical significance at the level of the sub-network of connections found to be reduced in functional connectivity, and not at the level of individual connections comprising this sub-network (Zalesky et al., 2010). However, to gain qualitative insight into the distribution of reduced connections and the brain regions most involved, we decomposed the sub-network based on 6 brain lobes. While reductions in FC were found between all six brain lobes, the majority of impaired connections were located between fronto-temporal, fronto-occipital, temporo-occipital and temporo-temporal lobes (Fig. 3a).

Next, we sought to identify the regions where the majority of connections were reduced. The temporal, occipital and frontal lobes were most implicated in reduced FC (Fig. 3a). As such, we applied the same method as previously described to obtain connectivity matrices for temporal, occipital and frontal subregions/AAL node components (Fig. 3b–d). The majority of reduced temporal lobe connections were located between Heschl's gyrus and the frontal lobe (Fig. 3b). The majority of reduced occipital lobe connections were located between the cuneus and the frontal lobe (Fig. 3c) and the majority of reduced frontal lobe connections were located between the paracentral lobule and the occipital lobe (Fig. 3d).

These subregions served as bilateral seeds for subsequent post-hoc seed-based analyses (see Supplementary material Fig. 2 and Tables I–III for results).

3.2. Network efficiency

Global efficiency was significantly reduced in TRS relative to controls ($p = 0.0015$), whereas local efficiency was significantly increased ($p = 0.0042$) (Fig. 2d). In a supplementary analysis, normalized efficiency was computed for binary graphs that were thresholded with respect to connection density, and results showed increased global and local efficiency in the TRS group (Supplementary material Fig. 2). Network efficiency was not significantly associated with medication (chlorpromazine equivalent dosage (global efficiency: $r = 0.20$, $p = 0.22$, local efficiency: $r = -0.22$, $p = 0.17$) or illness duration (global efficiency: $r = -0.06$, $p = 0.72$, local efficiency: $r = -0.05$, $p = 0.75$). Similarly, no significant associations between these clinical measures and functional connectivity were found. Illness duration and medication were therefore not included as nuisance covariates when testing for between-group differences.

Table 1
Demographic and clinical characteristics of participants.

	TRS patients (n = 42)	Controls (n = 42)	Between-group differences
Gender (male/female)	30/13	24/17	χ^2 (1, N = 85) = 0.98, p = 0.32
Age (year)	41.3 (10.0)	38.4(10.4)	$t(83) = 1.34$, p = 0.18
Illness duration (year)	17.9 (9.25)	–	–
Age of illness onset (year)	23.4 (6.6)		
IQ	86.1 (18.7)	111.2(13.6)	$t(75) = -6.70$, p = 0.000**
Education (years)	12.0 (0.55)	16.4(0.47)	$t(79) = -6.35$, p = 0.000**
GAF	45.9 (13.0)	79.5(10.6)	$t(79) = -12.79$, p = 0.000**
SOFAS	46.5 (14.8)	79.5(11.0)	$t(80) = -11.49$, p = 0.000**
Clozapine dosage (mg/day)	393.24 (24.6)	–	–
Chlorpromazine equivalent dosage (mg/day)	615.4 (55.84)	–	–
PANSS scores			
Positive	15.6 (6.58)	–	–
Negative	16.4 (5.18)	–	–
Disorganized	12.4 (4.09)	–	–
Excited	6.3 (2.66)	–	–
Depressed	8.3 (3.84)	–	–
Total	59.1 (13.1)	–	–

Note: TRS – Treatment-resistant schizophrenia; IQ – Intelligence Quotient; GAF – The Global Assessment of Functioning; SOFAS – Social and Occupational Functioning Assessment Scale; PANSS – Positive and Negative Syndrome Scale; mg – milligram.
* Significant $p < 0.05$.
** Significant $p < 0.001$.

3.3. Network properties and symptomatology/functioning

Significant associations between symptom severity/functioning and measures of FC strength and network efficiency were not evident.

4. Discussion

This study explored whole-brain resting-state functional connectivity (FC), and the efficiency of whole-brain networks in patients with schizophrenia who have not responded to antipsychotic treatment (treatment-resistant schizophrenia; TRS). We found widespread reductions in FC in the TRS group at the whole-brain level, particularly implicating temporal, occipital and frontal regions with follow-up analyses showing the subregions predominantly involved to be Heschl's gyri, cuneus and paracentral lobule. We also found the TRS group to show reduced global network efficiency and increased local network efficiency when compared to controls. An interesting question is whether the findings in our study of TRS provide evidence to a unique pattern of connectivity in comparison to the extant literature in schizophrenia.

4.1. Whole- brain functional connectivity

TRS individuals showed reduced FC in 3.4% of all possible connections, and no instance of increased connectivity relative to controls. The temporal lobes emerged as the regions most implicated, suggesting that temporal lobe dysconnectivity may play a central role in the pathophysiology of TRS. The temporal lobes have been consistently implicated in the biology of schizophrenia, with findings of volumetric reductions (Kuroki et al., 2006), hypoactivation (Takei et al., 2013) and disrupted structural (Minami et al., 2003) as well as increased and decreased FC (Meyer-Lindenberg et al., 2001). The current finding of decreased connectivity between frontal regions (areas involved in executive function and attention) and temporal regions (areas such as Heschl's and fusiform gyrus) suggests dysfunctional interactions between brain hubs that may lead to deficits in auditory and visual cognitive processes that rely on such connectivity. This fronto-temporal 'dysconnectivity' could in turn result in further cognitive impairment and the exacerbation of positive symptomatology that is characteristic of TRS, such as persistent auditory hallucinations and thought disorder. Evidence of reduced white matter integrity in fronto-temporal connections (Lim et al., 1999; Zalesky et al., 2011) and grey and white matter volumetric reductions in both frontal and temporal regions in schizophrenia cohorts (Walterfang et al., 2008; Wright et al., 2000) may provide a potential anatomical substrate for this reduction in FC. However additional research is necessary to determine whether this is the case in TRS.

Further, the observed reduced temporo-occipital (in particular the cuneus) FC in TRS may represent underlying biological disturbances that could be precipitating or exacerbating positive symptomatology, such as visual hallucinations. This however is speculative, as only 7 out of the 42 TRS patients were reported as experiencing visual hallucinations at the time of assessment. Therefore, although there may be a meaningful relationship between occipital FC and visual hallucinations,

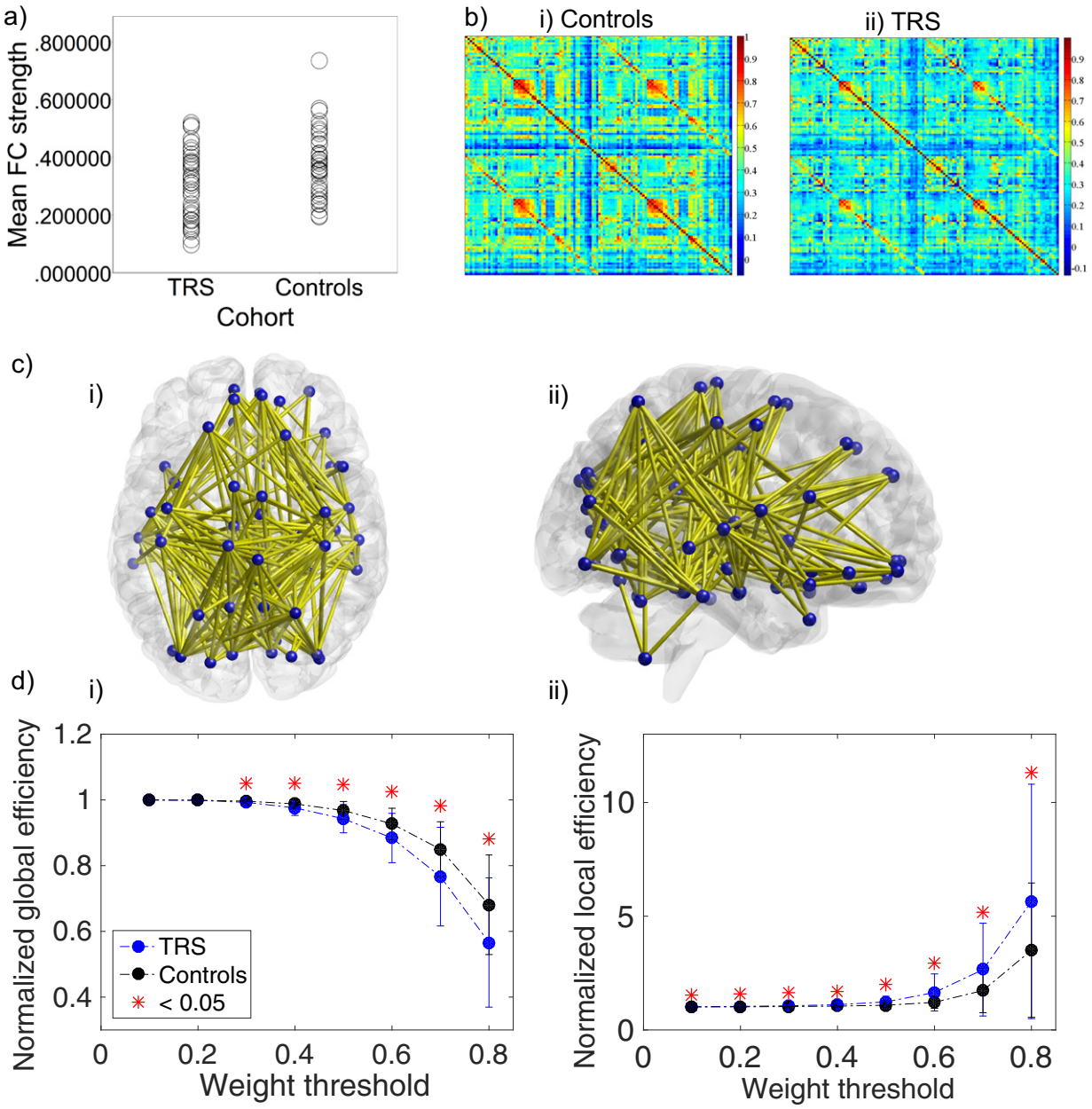

Fig. 2. Between group differences in whole-brain functional connectivity. a) Column scatter graph showing the group differences in mean FC across the whole brain ($p = 0.003$). Each circle represents a participant. Analyses were re-run excluding the control outlier, and results remained significant ($p = 0.005$). b) Averaged inter-regional FC matrix for i) controls and ii) patients. Individual 116 × 116 Fisher's r-to-z transformed connectivity matrices were obtained for each participant by calculating the Pearson correlation coefficient of time series between every pair of brain regions. A mean correlation matrix for patients (panel i) and controls (panel ii) was generated. Colour bars indicate correlation coefficients. c) Whole-brain connectivity differences between groups. These two figures represent i) the transverse and ii) sagittal view of the reduced functional connections (yellow lines, $n = 228$) between nodal pairs (blue spheres, $n = 70$) (TRS < Controls). The affected network was identified with the network-based statistic (NBS) using permutation testing ($n = 5000$) at a threshold of pFWE-cor < 0.001, with a *t*-statistic threshold of 4.0. d) Normalized global and local efficiency differences between groups accessed by weight thresholding. Each point along the blue (TRS) and black (controls) lines represents the averaged efficiency across participants of the group divided by the mean efficiency of the random networks generated for each group. The error bars denote one standard deviation. Red stars signify $p < 0.05$.

it is unlikely that the small fraction of participants experiencing these symptoms is driving the results. Future studies with greater numbers of patients experiencing visual hallucinations should endeavour to investigate this relationship. Reduced fractional anisotropy in white matter tracts connecting temporal and occipital regions (Bora et al., 2011; Ellison-Wright and Bullmore, 2009) have been found in schizophrenia and additionally, disturbances in white matter and corticocortical connectivity involving the cuneus (Moran et al., 2015) and temporo-occipital areas are seen in children with TRS treated with clozapine and their unaffected siblings (Zalesky et al., 2015). This raises an interesting question as to the nature of TRS; the fact that the occipital lobe and temporo-occipital connections are implicated in this study is somewhat unusual, given many of the changes seen in schizophrenia are often confined to frontal, cingulate and temporal regions. One possibility is that individuals that develop TRS may be experiencing early neurodevelopmental abnormalities, which is in line with the notion of an early 'hit' implicating more posterior brain regions (Cropley and Pantelis, 2014; Pantelis et al., 2005).

As previously mentioned, TRS has been speculated to be a separate sub-group of schizophrenia as opposed to an illness stage, making comparisons made to chronic schizophrenia literature potentially inaccurate. With this said, very little fMRI research has been conducted in

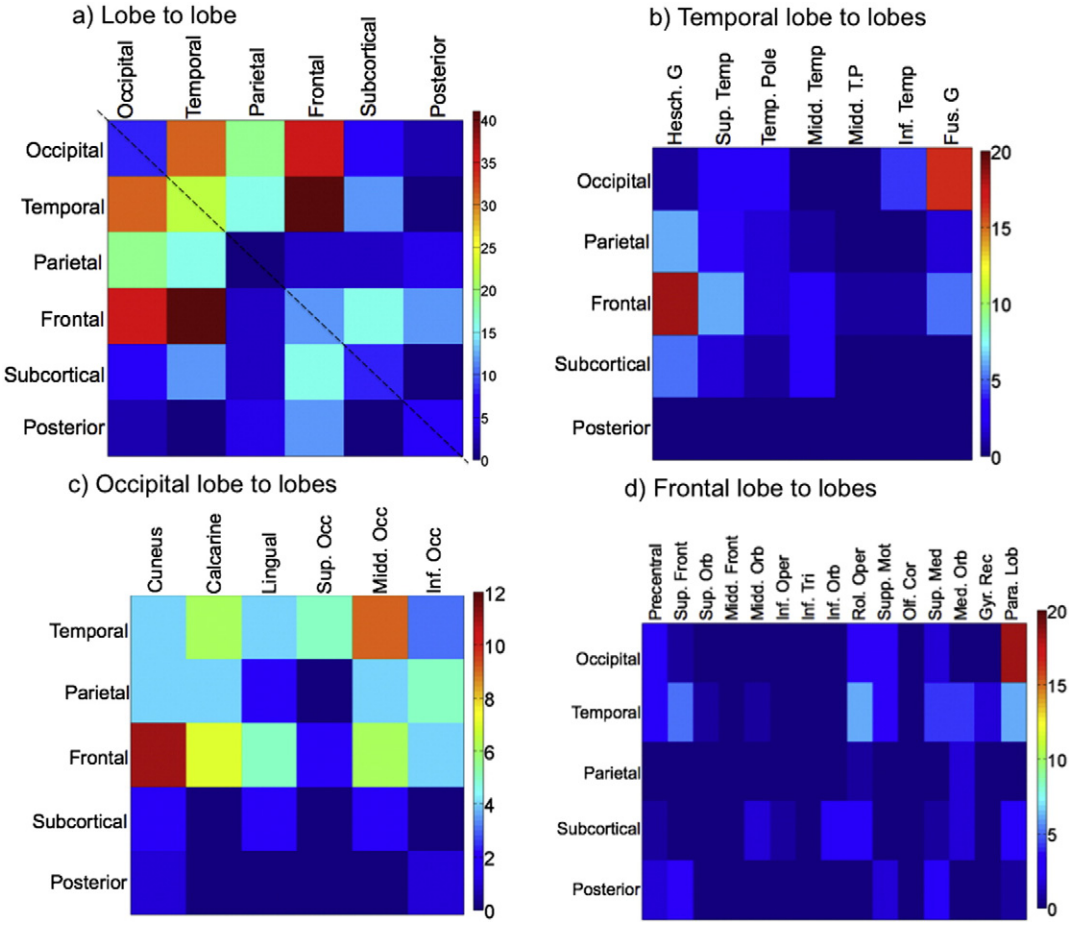

Fig. 3. Matrices showing the anatomical location and number of reduced functional connections between (a) the six brain lobes, b) temporal lobe subregions (c) occipital lobe subregions and (d) frontal lobe subregions and remaining brain lobes. The colour bar represents the raw number of connections found to be reduced in connectivity strength in the TRS group. Hesch. G = Heschl's gyrus; Sup. Temp = Superior temporal; Temp. Pole = Temporal pole; Midd. Temp = Middle temporal; Midd. T. P = Middle temporal pole; Inf. Temp = Inferior temporal; Fus. G = Fusiform gyrus; Sup. Occ = Superior occipital; Midd. Occ = Middle occipital; Inf. Occ = Inferior occipital; Sup. Front = Superior frontal; Sup. Orb = Superior orbital; Midd. Front; Middle frontal; Midd. Orb = Middle orbital; Inf. Oper = Inferior operculum; Inf. Tri = Inferior triangularis; Inf. Orb = Inferior orbital; Rol. Oper = Rolandic operculum; Supp. Mot = Supplementary motor; Olf. Cor = Olfactory cortex; Sup. Med = Superior medial; Med. Orb = Medial orbital; Gyr. Rec = Gyrus Rectus; Para. Lob = Paracentral lobule.

TRS populations, limiting the amount of studies with which we can compare the current findings to. However, those that have investigated TRS rs-FC generated mixed results, with one study reporting reduced FC involving temporal regions (Vercammen et al., 2010) while a later study found increased FC between the bilateral temporal regions and decreased FC between other brain regions (Lee et al., 2002). Identifying a FC profile that is unique to TRS is beyond the scope of this study given our inability to compare TRS with treatment responsive schizophrenia. However, in contrast with previous rs-FC studies in other schizophrenia groups, our results showed the occipital lobe to be predominantly involved in reduced FC, indicating that occipital 'dysconnectivity' may play a larger role in the pathophysiology of this cohort. The current findings in conjunction with the previous results do not necessarily point towards specific predictive markers of TRS, but represent a step towards identifying the neurobiological mechanisms that are underlying this resistance to antipsychotic treatment.

4.2. Network efficiency

To our knowledge, this study was the first to explore network efficiency in a TRS cohort. Our finding of decreased global efficiency in the TRS group is in line with previous functional (Hadley et al., 2016; Lo et al., 2015; Lynall et al., 2010) and structural (Zalesky et al., 2011) findings in schizophrenia cohorts and indicates that topologically, TRS is associated with reduced global network integration that is not a result of the widespread reduction in FC strength. Interestingly, however, the TRS group showed increased local efficiency which has also been previously reported in schizophrenia groups (Hadley et al., 2016; Lynall et al., 2010). Increased local efficiency coupled with decreased global efficiency suggests that hub-to-hub connections are preferentially affected in TRS. Specifically, when hub-to-hub connections are eliminated, global efficiency is reduced in TRS relative to controls, as these connections facilitate global integration between disparate modules. This may suggest a failure to integrate information among different brain hubs, and this disruption in communication of spatially separate brain regions could result in a distortion of thoughts and perceptions (Cao et al., 2015). Moreover, when hub-to-hub connections are eliminated, local efficiency is increased in TRS relative to controls (once normalized to degree-matched random networks). This may be because the TRS networks comprise a greater proportion of peripheral, intra-modular connections, which support increased local efficiency.

This increase in local efficiency is in line with the hypothesis that individuals with schizophrenia show some neurobiological advantages that have enabled the disorder to exist throughout generations (Lynall et al., 2010). Higher local efficiency means that more alternative paths exist between two given regions in the event that one path (or connection) is destroyed as a result of brain trauma or disease, resulting in a more clustered topology with a greater robustness to random attack (Lynall et al., 2010). However, an increase in 'back-up' alternative connections may be metabolically costly and inefficient, which could in

part explain the reduced FC and global efficiency. An excess of local connections in the absence of intact long-range (global) connections might reflect the inefficiency of poorly integrated content such as hallucinations and delusions that are common symptoms of TRS (Su et al., 2015).

4.3. Network properties and symptomatology/functioning

In terms of relating our network findings to TRS symptomatology, the present study found no evidence of association. This however, does not contradict previous findings of relationships between abnormal network properties and symptomatology in schizophrenia and TRS cohorts, but instead may represent a downstream ceiling effect of brain connectivity on symptom severity due to prolonged illness duration and long-term medication effects. Future studies should further explore whether and how reduced FC and network efficiency is associated with clinical heterogeneity of symptoms. Longitudinal research is also needed to establish the extent to which antipsychotic medication and illness progression influences symptomatology and brain connectivity and topology, and whether this varies for those with TRS.

4.4. Limitations and future directions

Given the chronicity of this TRS sample, it is difficult to disentangle whether and to what extent our findings may be influenced by the effects of illness duration and/or prolonged antipsychotic use, both of which have been linked with structural (Navari and Dazzan, 2009) and functional changes in the brain (Liu et al., 2008). Neither medication dosage nor illness duration correlated with FC strength or network efficiency in the present cohort; however, the potential effect of these variables on results should be addressed in future research with samples with greater variance in these properties. A further limitation of this study is the inability to compare TRS individuals to treatment responsive individuals with schizophrenia. Comparing TRS with healthy controls limits the inferences that can be made, as differences found between groups cannot necessarily be interpreted as markers of 'treatment resistance' but instead may reflect anomalies common to schizophrenia in general (Nakajima et al., 2015).

In summary, TRS represents the most chronic form of psychotic illness, with patients often experiencing worse clinical outcome, lifelong social and occupational dysfunction and a poorer quality of life (Pantelis and Barnes, 1996). An important question that remains unanswered is whether TRS represents a severe illness stage of schizophrenia or whether it is instead best viewed and treated as a distinct neurobiological sub-type that responds to fundamentally different treatments in comparison with treatment-responsive schizophrenia (Wimberley et al., 2016). Although the current results have not disentangled the underlying rs-mechanism that signifies treatment-resistance, they contribute to a growing profile that will hopefully one day elucidate key predictors of TRS. Thus, to better understand the underlying mechanisms of TRS and in turn progress the development and implementation of alternative therapeutic strategies, further neuroimaging and neurogenetic work is crucial.

The results of the present study indicate that TRS is associated with widespread reductions in rs-FC and abnormal network efficiency, and for this cohort, there is little evidence of a relationship between these network properties and symptomatology/functioning. Further research needs to be undertaken to compare TRS individuals with non-TRS individuals longitudinally in order to clarify the varying underlying neural mechanisms that contribute to clinical heterogeneity within the schizophrenia spectrum.

Contributors

Author Zalesky designed the functional connectivity protocol and was imperative to the methodology and analysis of the neuroimaging data. Author Seguin assisted in the design and execution of the graph theory section. Author Pantelis, author Phassouliotis and author Everall were imperative to the design, recruitment and execution of the study. Author Whittle, author Bousman and author Bartholomeusz assisted in the statistical design of the study. Author Ganella performed all the neuroimaging and statistical analyses and wrote the manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of interest

The authors declare they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2016.12.008.

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Supplementary Material

Figure 1. Craddock template verification: i) Transverse and ii) Sagittal view of the functional connections found to be reduced in the TRS cohort (denoted by the yellow lines, n=237) between nodal pairs (denoted by the blue spheres, n=72) (TRS < Controls). The affected network was identified with the network-based statistic (NBS) using permutation testing (n=5000) at a threshold of pFWE-cor <0.001, with a t-statistic threshold of 3.2.

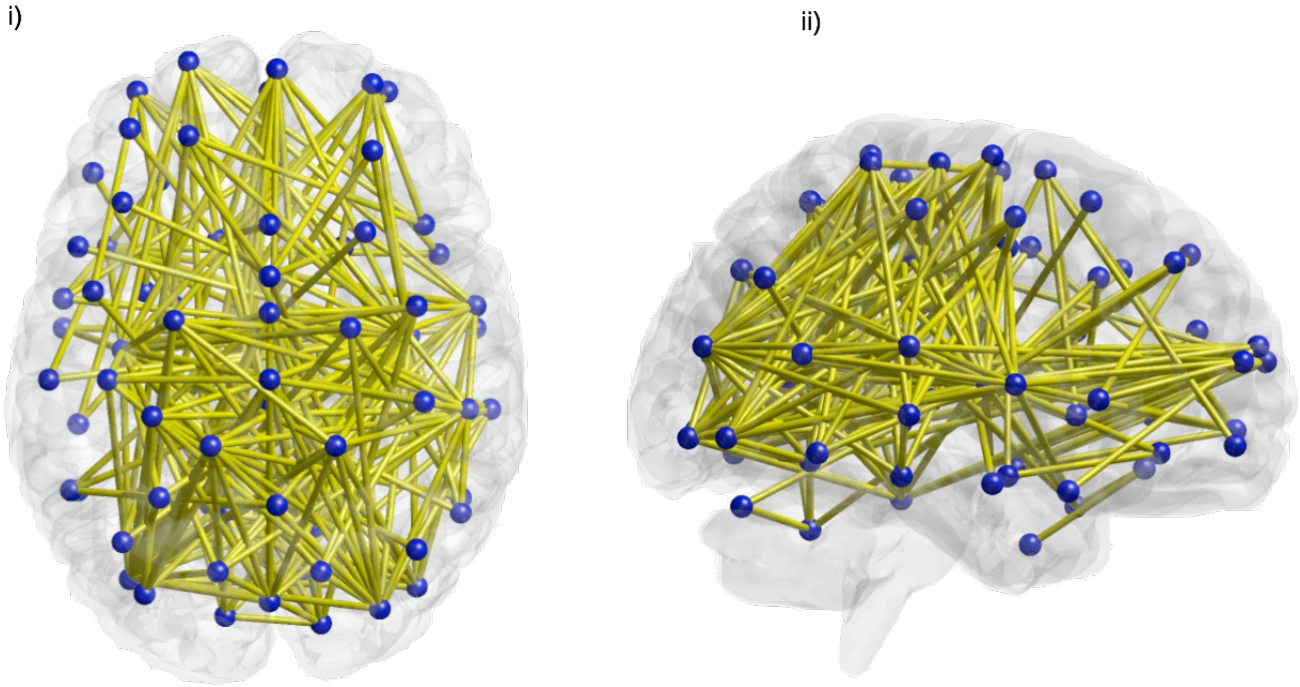
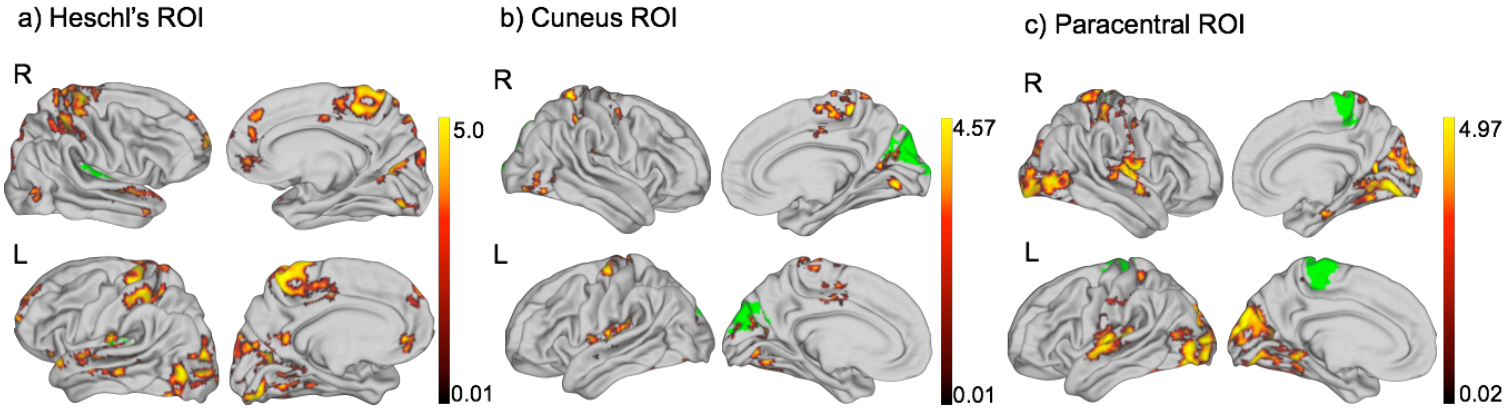
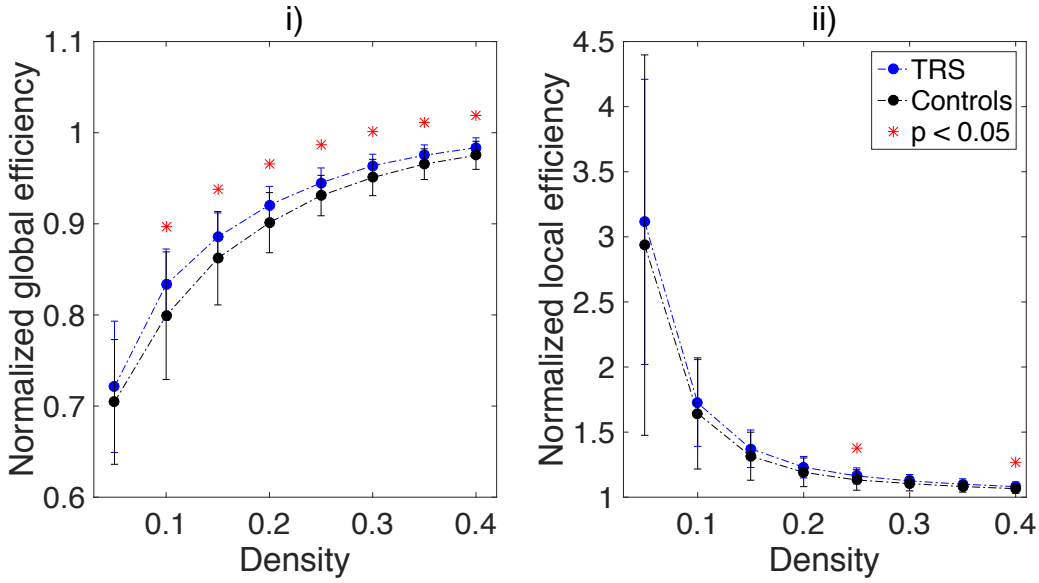


Figure 2. Seed-based maps showing clusters of voxels whose correlation with a) Heschl's gyrus, b) cuneus and c) paracentral lobule significantly differed between groups (TRS < Controls). A bilateral binary mask was created to isolate the subregions as ROI's to perform ROI to whole brain voxel-wise connectivity. Pearson correlation coefficients were computed between the voxel-averaged signal for the ROI and all other voxels comprising cortical and subcortical gray matter. To ensure statistical testing did not include nuisance signals from white matter and cerebrospinal fluid, a grey matter mask was used. Green regions represent the ROI's. Connectivity maps were converted to Fisher's z-scores to improve normality of the data and group differences were calculated using permutation testing (n=5000) at a threshold of pFWE-cor<0.05, with a t-statistic threshold of 3.5. Colour bars indicate T score. See supplementary material Table I-III for more information.



Note: TRS = Treatment-resistant schizophrenia; FWE-cor = Family-wise error rate-corrected.

Figure 3. Normalized global and local efficiency differences between groups accessed by density thresholding. Each point along the blue (TRS) and black (controls) lines represents the averaged efficiency across participants of the group. The error bars denote one standard deviation. Red stars mark significant differences (p-value < 0.05). Density thresholding was performed by keeping only the strongest connections necessary to achieve a certain density and binarising the resulting network.



Note: TRS = Treatment-resistant schizophrenia

Table I. Brain regions showing connectivity differences with the bilateral Heschl's gyrus between healthy controls and patients with TRS

Brain regions	Peak T value	P value	Cluster Number/size	(x,y,z)
Bilateral pre/post central gyrus	5.88	0.001	10(4527)	41,51,63
Bilateral fusiform/lingual gyrus	6.51	0.001	9(4452)	58,30,36
Left superior/middle temporal gyrus/OFC	6.65	0.002	8(947)	76,61,31
Left frontal pole	5.3	0.004	7(517)	53,98,36
Right superior temporal gyrus	5.04	0.005	6(311)	17,69,21
Bilateral medial frontal cortex	4.82	0.007	5(234)	51,83,31
Right frontal pole	5.99	0.011	4(175)	36,97,30
Bilateral lateral occipital cortex	4.16	0.030	3(110)	21,27,38

Table II. Brain regions showing connectivity differences with the cuneus between healthy controls and patients with TRS

Brain regions	Peak T value	P value	Cluster Number/size	(x,y,z)
Left Heschl's gyrus	5.87	0.002	19(660)	69,54,43
Right precuneus/pre and postcentral gyrus/cingulate gyrus	5.6	0.004	18(458)	42,44,65
Left lingual gyrus	5.04	0.009	17(239)	52,25,35
Left postcentral gyrus	4.89	0.01	16(228)	63,55,68
Right cuneual cortex	4.89	0.012	15(180)	37,28,45
Left planum temporale/Heschl's gyrus	4.43	0.013	14(173)	19,53,43
Left superior temporal gyrus	5.9	0.013	13(172)	17,47,45
Left precentral gyrus	5.09	0.018	12(146)	51,49,75

Table III. Brain regions showing connectivity differences with the bilateral paracentral lobule between healthy controls and patients with TRS

Brain regions	Peak T value	P value	Cluster Number/size	(x,y,z)
Bilateral pre/post central gyrus	5.91	0.001	9(7892)	54,20,51
Bilateral fusiform/lingual gyrus	6.11	0.001	8(1619)	68,58,39
Left superior/middle temporal gyrus/OFC	5.32	0.001	7(1406)	17,59,43
Left frontal pole	4.99	0.003	6(522)	31,42,69
Right superior temporal gyrus	4.84	0.009	5(213)	70,52,54
Bilateral medial frontal cortex	4.61	0.023	4(123)	27,56,68
Right frontal pole	5.03	0.033	3(102)	17,59,60
Bilateral lateral occipital cortex	4.39	0.036	2(100)	55,42,64

CHAPTER 4:

Risk and resilience brain networks in treatment-resistant schizophrenia

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Risk and resilience brain networks in treatment-resistant schizophrenia

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ABSTRACT

Background: Genes, molecules and neural circuits that are associated with, or confer risk to developing schizophrenia have been studied and mapped. It is hypothesized that certain neural systems may counterbalance familial risk of schizophrenia, and thus confer resilience to developing the disorder. This study sought to identify resting-state functional brain connectivity (rs-FC) representing putative risk or resilience endophenotypes in schizophrenia.

Methods: Resting-state functional magnetic resonance imaging (rs-fMRI) was performed in 42 individuals with treatment resistant schizophrenia (TRS), 16 unaffected first-degree family members (UFM) and 42 healthy controls. Whole-brain rs-FC networks were mapped for each individual and analysed graph theoretically to identify network markers associated with schizophrenia risk or resilience.

Results: The ~900 functional connections showing between-group differences were operationalized as conferring: i) resilience, ii) risk, or iii) precipitating risk and/or illness effects. Approximately 95% of connections belonged to the latter two categories, with substantially fewer connections associated with resilience. Schizophrenia risk primarily involved reduced frontal and occipital rs-FC, with patients showing additional reduced frontal and temporal rs-FC. Functional brain networks were characterized by greater local efficiency in UFM, compared to TRS and controls.

Conclusions: TRS and UFM share frontal and occipital rs-FC deficits, representing a 'risk' endophenotype. Additional reductions in frontal and temporal rs-FC appear to be associated with risk that precipitates psychosis in vulnerable individuals, or may be due to other illness-related effects, such as medication. Functional brain networks are more topologically resilient in UFM compared to TRS, which may protect UFM from psychosis onset despite familial liability.

1. Introduction

Schizophrenia has a strong genetic component, with the most prominent risk factor for developing the disorder being family history (Gottesman and Gould, 2003; Kendler and Neale, 2010). Studying unaffected relatives of individuals with schizophrenia can therefore offer insight into the heritable pathophysiology of the disorder, independent of factors that often confound studies in patients, such as illness progression and chronic antipsychotic use (Braff et al., 2007). A number of structural brain alterations are shared between schizophrenia patients and their unaffected family members (UFM) representing candidate endophenotypes (Moran et al., 2013; Turetsky et al., 2007), such as cortical thinning (Goghari et al., 2007; Gogtay et al., 2007; Yang et al., 2010), reduced morphological covariance (Zalesky et al., 2015), whole brain (McIntosh et al., 2011; Thermenos et al., 2013) and subcortical

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volume reductions (Peper et al., 2007; Thermenos et al., 2013). Less understood, however, is the nature of structural and functional brain connectivity abnormalities in UFM. Decreased resting-state functional connectivity (rs-FC) is commonly reported in schizophrenia patients, although increased rs-FC is also described (for review, see Fitzsimmons et al., 2013). Similarly, studies in UFM have generated mixed results, with some findings showing increased rs-FC relative to controls (Jang et al., 2011; Jukuri et al., 2013; van Buuren et al., 2012; Whitfield-Gabrieli et al., 2009), while others report reduced rs-FC (A. Fornito et al., 2013; Jang et al., 2011; Jukuri et al., 2015; Khadka et al., 2013; Liu et al., 2012; Meda et al., 2012; Sole-Padulles et al., 2016). On the whole, rs-FC is predominantly reduced in schizophrenia patients (Fornito et al., 2012), and the functional brain networks most affected often show milder rs-FC reductions in UFM (Wang et al., 2015). Aberrant rs-FC networks shared between patients and UFM may therefore represent a marker of genetic vulnerability to schizophrenia, rather than solely the result of illness duration, medication and/or other secondary environmental factors (Repovs et al., 2011).

Alternatively, rs-FC alterations that are unique to UFM *and* absent or moderated in affected relatives and the general population might be hypothesized to represent putative markers of resilience to schizophrenia, and counterbalance familial liability. Resilience biomarkers have not been extensively studied in schizophrenia, with resilience in psychiatry traditionally broached in terms of psychological response to stress and trauma (Feder et al., 2009; Russo et al., 2012). Recent evidence suggests that functional brain networks in UFM show increased resilience to pathological disruptions, compared to schizophrenia patients and controls (Lo et al., 2015). Similarly, UFM show resilience in that they recover from developmental delays in structural connectivity (Chakravarty et al., 2015; Zalesky et al., 2015). Resilience endophenotypes inferred from rs-FC have also been reported in depression (Peterson et al., 2014). These previous studies motivate investigation of functional brain networks associated with resilience in schizophrenia.

Here, we perform resting-state functional magnetic resonance imaging (fMRI) in individuals with treatment-resistant schizophrenia (TRS), UFM and healthy controls, with the aim of identifying functional brain networks associated with schizophrenia risk or resilience. We operationalize resilience as functional connections or functional network properties that are uniquely present (or absent) in UFM individuals, whereas network properties that are shared between TRS and UFM (but not evident in the general population) are considered risk markers. We consider TRS patients in this study to ensure a homogeneous clinical phenotype (Jablensky, 2006), and thereby maximize the reproducibility of our findings. We and others have found that TRS is associated with widespread abnormal rs-FC (Ganella et al., 2017; Vercammen et al., 2010; White et al., 2016; Wolf et al., 2011) and we hypothesize that investigating UFM of TRS patients will provide insight into functional networks associated with schizophrenia risk and resilience. Specifically, we hypothesize both the TRS and UFM groups to show reduced rs-FC and network efficiency relative to controls, albeit to a lesser extent in UFM.

2. Methods

2.1. Participants

Forty-two TRS individuals (mean age 41.3 ± 10.0, 30 males) were recruited from inpatient and outpatient clinics located in Melbourne, Australia, as previously described (Ganella et al., 2017). TRS was defined as at least two unsuccessful trials (4–10 weeks) of two or more different antipsychotic types (dosage equivalent to 1000 mg/d chlorpromazine) within the last 5 years, with a Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) total score ≥ 90 and currently taking clozapine (Kane et al., 1988; Suzuki et al., 2012). The study consisted of 16 UFM first-degree relatives of TRS patients (mean age 57.5.4 ± 11.7, 2 males) and 42 (unrelated) healthy controls (mean age 38.4 ± 10.4, 24 males) who were recruited from the general community. Ten UFM had a biological first-degree relative with TRS included in this study, the remaining 6 UFM had a biological first-degree relative with TRS who either did not participate in the MRI component (n = 5), or participated but was excluded due to excessive movement at the time of scanning (n = 1).

All participants were administered the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) to confirm diagnosis of schizophrenia and to rule out current or past psychiatric illness in healthy controls. Clinical symptoms were assessed using the PANSS, and all participants were evaluated using the Global Assessment of Functioning (GAF) (Hall and Parks, 1995) and the Social and Occupational Functioning Assessment Scale (SOFAS) (Goldman et al., 1992). The study was approved by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069); and all participants provided written informed consent prior to participation.

2.2. Imaging data acquisition

Magnetic resonance images were acquired on a Siemens Avanto 3T Magnetom TIM Trio scanner. T1-weighted images were acquired using an optimized Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence. The sequence parameters were: 176 sagittal slices of 1 mm thickness without gap, field of view (FOV) = 250 × 250 mm^2, repetition time (TR) = 1980 ms, echo time (TE) = 4.3 ms, flip angle = 15°, using an acquisition matrix of 256 × 256 resulting in a final reconstructed voxel resolution of 0.98 × 0.98 × 1.0 mm^3. Resting-state fMRI was acquired using a $T2^*$-weighted echo-planar imaging sequence (TE = 40 ms; TR = 2.4 s; voxel dimensions = 3.3 × 3.3 × 3.5; matrix size = 64 × 64). Data was acquired for 8 min, resulting in 200 volumes.

2.3. fMRI data preprocessing

Data preprocessing was performed using FSL (FMRIB Software Library, www.fmrib.ox.ac.uk) and SPM8 (http://www.fil.ion.ucl.ac.uk/spm). For each subject, echo-planar images were slice-time corrected, realigned to the mean functional image to correct for motion, coregistered to the structural T1-weighted scan via rigid-body registration and then spatially normalized by non-linear registration to the Montreal Neurological Institute (MNI) 152 template with 2-mm resolution. Data was spatially smoothed using a Gaussian kernel of full width at half maximum 4 mm and bandpass filtered (0.01–0.1 Hz).

Head motion was controlled with the Friston 24-parameter model (Friston et al., 1996) and signals from white matter and the ventricles were regressed to account for physiological noise. The global signal was not regressed due to ongoing controversy surrounding whether this step is warranted when mapping rs-FC in schizophrenia probands (Yang et al., 2014). Given that measures of rs-FC may be influenced by head motion (Power et al., 2012), each individual's movement during scanning was quantified using framewise displacement (FD) (Power et al., 2013). FD is a compressed single index calculated from derivatives of the six rigid-body realignment parameters. Volumes exceeding a FD of 0.5 mm, a commonly used threshold (Power et al., 2012) were eliminated, otherwise known as scrubbing.

2.4. Functional network mapping and operationalization of risk and resilience

For each individual, a whole-brain resting-state functional network was mapped using established methods (Fornito et al., 2016). In brief, regionally-averaged fMRI signals were determined for the $N = 116$ region automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002), and the $N = 360$ region Glasser atlas (Glasser et al., 2016) (see Supplementary material). For each individual, regionally-

averaged fMRI signals were then correlated in a pairwise manner using Pearson correlation, resulting in a $N \times N$ rs-FC matrix.

Analysis of covariance (ANCOVA) with age and gender as covariates was used to test the null hypothesis of equality in mean rs-FC between the three groups: TRS, UFM and healthy controls. The null hypothesis was tested independently for each of the $N(N-1)/2$ connections and the network-based statistic (NBS) (Zalesky et al., 2010) was used to control the familywise error rate. The NBS was applied with a primary *F*-statistic threshold of 5 ($p = 0.01$) and 10,000 permutations were generated to construct an empirical null distribution for the size of the maximal sub networks. Functional connections comprising sub-networks for which the null hypothesis was rejected for a familywise error rate of 0.05 were subjected to post hoc *t*-tests. Specifically, post-hoc *t*-tests were performed to assess the null hypothesis of equality between: i) TRS and healthy controls, and ii) UFM and healthy controls. Based on these two post-hoc *t*-tests, connections that significantly differed between the three groups were classified into three mutually exclusive categories as follows: **Risk** was operationalized as reduced (or increased) connectivity in *both* TRS and UFM, relative to healthy controls; that is, risk required both post-hoc *t*-tests to reach significance in the *same* direction. **Precipitating risk** and/or **illness effects** were operationalized as reduced (or increased) connectivity in TRS relative to healthy controls, with no significant difference between UFM and controls; that is, precipitating risk required significance of the post-hoc *t*-test involving TRS, but not the one involving UFM. **Resilience** was operationalized as reduced (or increased) connectivity in UFM relative to controls, and either no significant difference between TRS and controls or the opposite difference in TRS compared to controls. Under this categorization, resilience can be conceptualized as absence or presence of a functional connection that is unique to UFM.

2.5. Graph theoretic analyses

Network resilience was quantified using *local efficiency*, a graph measure that characterizes how well the neighbors of a given brain region remain connected after that region and its associated connections are removed from the network (Latora and Marchiori, 2001). *Global efficiency*, a measure of network integration that captures a system's ability to exchange information in a parallel manner, was also investigated (Bullmore and Sporns, 2012). Local and global efficiency were computed for graphs that were binarized with respect to a series of connection weight thresholds ranging between 0.1 and 0.6 applied to rs-FC matrices of each individual (Rubinov and Sporns, 2010). The area under curve (AUC) was used as a summary statistic of local and global efficiency (Lynall et al., 2010).

Connection weight thresholding resulted in binary graphs that were not matched in terms of connection density across individuals (lower connection density was evident in TRS and UFM compared to healthy controls). To ensure between-group differences in connection density did not manifest as trivial between-group differences in local and global efficiency, two strategies were employed: i) efficiency measures were normalized with respect to degree and density preserving random graphs, mitigating dependencies on connection density to some extent (Maslov and Sneppen, 2002; van den Heuvel et al., 2017); ii) efficiency was also separately computed within network modules (modules were better matched across the three groups in connection density). Modular decomposition of the network was performed using the Fast Louvain's Algorithm (Blondel et al., 2008) (see Supplementary Material for further information). ANCOVA was used to test the null hypothesis of equality in the AUC of local and global efficiency between the three groups. This was repeated independently for the four network modules that were identified.

Figures were generated using BrainNet Viewer (Xia et al., 2013), NeuroMArVL (http://immersive.erc.monash.edu.au/neuromarvl/) and in-house Matlab scripts.

2.6. Functional connectivity and symptomatology/functioning

Pearson correlation was used to test for an association between rs-FC and network efficiency with the five PANSS subscales (positive, negative, excitement, disorganized, depressed) and total score (Wallwork et al., 2012) in the TRS group, and with GAF and SOFAS scores for the three groups.

3. Results

Demographic and clinical information is shown in Table 1. UFM were significantly older and comprised a significantly greater proportion of females than TRS and healthy controls. Gender, age and the square of age were included as covariates because these factors are known to have a significant influence on functional brain connectivity (Gong et al., 2011) and their inclusion can thus account for variance that is not explained by between-group differences. To further assess the effect of gender, we conducted a secondary analysis investigating only females and found comparable rs-FC results despite excluding over 50% of the sample (Supplementary Material). For brevity, here we only present results pertaining to the AAL atlas. Results were reproduced for the Glasser atlas and relegated to Supplementary material. Sensitivity analyses excluding the 6 UFM participants who did not have an affected relative in the study can also be found in Supplementary Material.

3.1. Functional connections associated with risk and resilience

Group-averaged rs-FC matrices are shown in Fig. 1a for TRS, UFM and controls. The null hypothesis of equality in mean rs-FC between the three groups was rejected for a total of 894 connections (13% of all possible connections). Post-hoc *t*-tests were performed on each significant connection to classify them into three mutually exclusive categories: i) risk, ii) precipitating risk and/or illness effects and iii) resilience. Fig. 1b shows a two-dimensional representation of this classification scheme. The majority of connections ($n = 545$, 61% of significant connections) reside within the lower-left risk quadrant (dark red), which represents reduced rs-FC in both TRS and UFM. These connections represent a rs-FC marker of familial vulnerability in schizophrenia. A smaller group of connections ($n = 302$, 34%) resides within the precipitating risk/illness effect quadrant (light red), which represents reduced rs-FC in TRS, but not UFM. In contrast, far fewer connections ($n = 47$, 5%) reside within the resilience quadrants (blue).

We next sought to anatomically localize connections comprising the quadrants in Fig. 1 to distinct regions and brain lobes. To this end, Fig. 2a shows circular connectograms for each of the three most densely populated quadrants in Fig. 1. For each connectrogram, the proportion of connections originating from each brain lobe was quantified (Fig. 2b). Fig. 2b shows that reduced rs-FC associated with risk predominantly originates from temporal (in particular the fusiform gyri; 26%), and occipital regions (predominantly the middle occipital lobe; 27%), whereas precipitating risk and illness effects are associated with *additional* rs-FC reductions that are mainly localized to frontal (particularly the paracentral lobule and rolandic operculum; 23%) and temporal regions (predominantly the Heschl's gyri; 29%). The 47 connections classified as resilience markers were mainly found between temporal (particularly the middle temporal pole) and subcortical regions (predominantly the posterior cingulum). Connections were mainly between lobes (interlobar) as opposed to being within the same lobe (intra-lobar) (Fig. 2c).

3.2. Graph theoretic analysis of resilience

The null hypothesis of equality in AUC between TRS, UFM and healthy controls was rejected for both normalized local ($F = 5.84$, $p = 0.0033$) and global efficiency ($F = 6.27$, $p = 0.0030$) (Fig. 3a). Compared with controls, local efficiency was significantly

Table 1
Demographic and clinical characteristics (means with standard deviations in parentheses).

	TRS (n = 42)	Controls (n = 42)	UFM (n = 16)	TRS vs Controls	UFM vs controls
Gender (male/female)	30/13	24/17	2/14	$\chi^2(1,N = 84) = 0.98$, p = 0.32	$\chi^2(1,N = 58) = 10.30$, p = 0.001*
Age (year)	41.3(10.0)	38.4(10.4)	57.5(11.7)	t(83) = 1.34, p = 0.18	t(56) = 6.05, p = 0.000**
Illness duration (year)	17.9(9.25)	–	–	–	–
Age of illness onset (year)	23.4(6.6)	–	–	–	–
IQ	86.1(18.7)	111.2(13.6)	112(14.9)	t(75) = −6.70, p = 0.000**	t(49) = 0.17, p = 0.86
Education (years)	12.0(0.55)	16.4(0.47)	15.81(3.9)	t(79) = −6.35, p = 0.000**	t(54) = −0.75, p = 0.46
GAF	45.9(13.0)	79.5(10.6)	76.8(9.1)	t(79) = −12.79, p = 0.000**	t(56) = −0.044, p = 0.97
SOFAS	46.5(14.8)	79.5(11.0)	79.3(9.9)	t(80) = −11.49, p = 0.000**	t(56) = −0.90, p = 0.37
Clozapine dosage (mg/day)	393.24(24.6)	–	–	–	–
Chlorpromazine equivalent dosage (mg/day)	615.4(55.84)	–	–	–	–
PANSS scores					
Positive	15.6(6.58)	–	–	–	–
Negative	16.4(5.18)	–	–	–	–
Disorganized	12.4(4.09)	–	–	–	–
Excited	6.3(2.66)	–	–	–	–
Depressed	8.3(3.84)	–	–	–	–
Total	59.1(13.1)	–	–	–	–

TRS – treatment-resistant schizophrenia; UFM – unaffected family members; IQ – intelligence quotient; GAF - The Global Assessment of Functioning; SOFAS - Social and Occupational Functioning Assessment Scale; PANSS - Positive and Negative Syndrome Scale; mg – milligram.
* Significant p < 0.05.
** Significant p < 0.001.

increased in UFM (p = 0.0180) and TRS (p = 0.0020) (Fig. 3a), however, there was no significant difference between UFM and TRS (p = 0.064). Compared with controls, global efficiency was significantly reduced in UFM (p < 0.001) and TRS (p < 0.001), however there was no significant difference between UFM and TRS (p = 0.10) (Fig. 3a). Therefore, both TRS and UFM were significantly more resilient to local disruptions in rs-FC compared to healthy controls, however this resilience was at the expense of a significant reduction in global network efficiency.

To ensure better matching of connection density across the three groups, normalized local efficiency was analyzed separately for the four network modules: attention/salience, default-mode, visual and cerebellar (Fig. 3b). Most between-group connectivity differences were inter-modular and, thus, constraining graph analysis to each module enabled disambiguation of topological between-group differences from differences in connection density. The null hypothesis of equality in AUC between TRS, UFM and healthy controls was rejected for two of

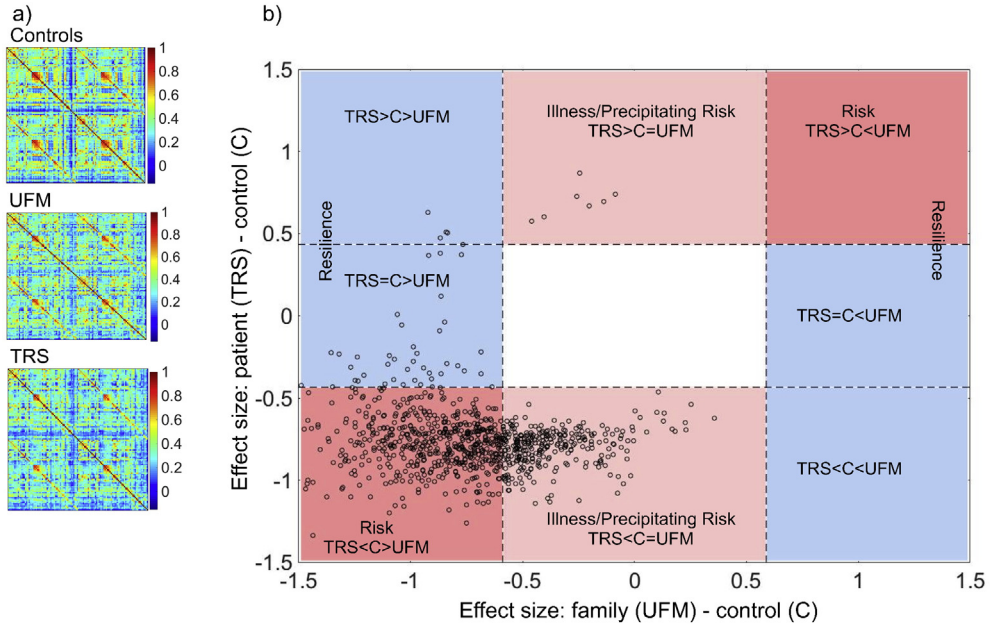

Fig. 1. a) Group-averaged 116 × 116 rs-FC matrices for control, UFM and TRS groups. Connectivity matrix elements contain r-to-z transformed, group-averaged correlation coefficients for 6670 pairs of regions. Matrix elements in the upper sub-block contain left hemisphere connections, those in the lower sub-block represent the right hemisphere and the off-diagonal sub-block contains inter-hemispheric connections. The prominent off-diagonal elements correspond to contralateral homologous regions. b) Two-dimensional representation of the risk and resilience classification scheme. Each open circle represents a connection for which the null hypothesis of equality in mean rs-FC between TRS, UFM and control groups was rejected (p < 0.05, familywise error corrected for 6670 tests). The horizontal axis is the effect size for post-hoc *t*-tests comparing UFM with healthy controls and the two dashed vertical lines correspond to α = 0.05 cut-off thresholds for the *t*-test. Analogously, the vertical axis and two horizontal dashed lines relate to post-hoc *t*-tests comparing TRS and healthy controls. The two-dimensional space is subdivided into 9 quadrants, with the central quadrant (white) representing cases where both post-hoc *t*-tests were not significant. At least one of the post-hoc *t*-tests was significant for the other 8 quadrants. The significance and direction of the post-hoc *t*-tests determined whether connections were assigned to a quadrant associated with i) risk (red quadrants), ii) resilience (blue quadrants), or iii) illness/precipitating risk (pink quadrants). TRS = treatment-resistant schizophrenia; UFM = unaffected family members; C = controls.

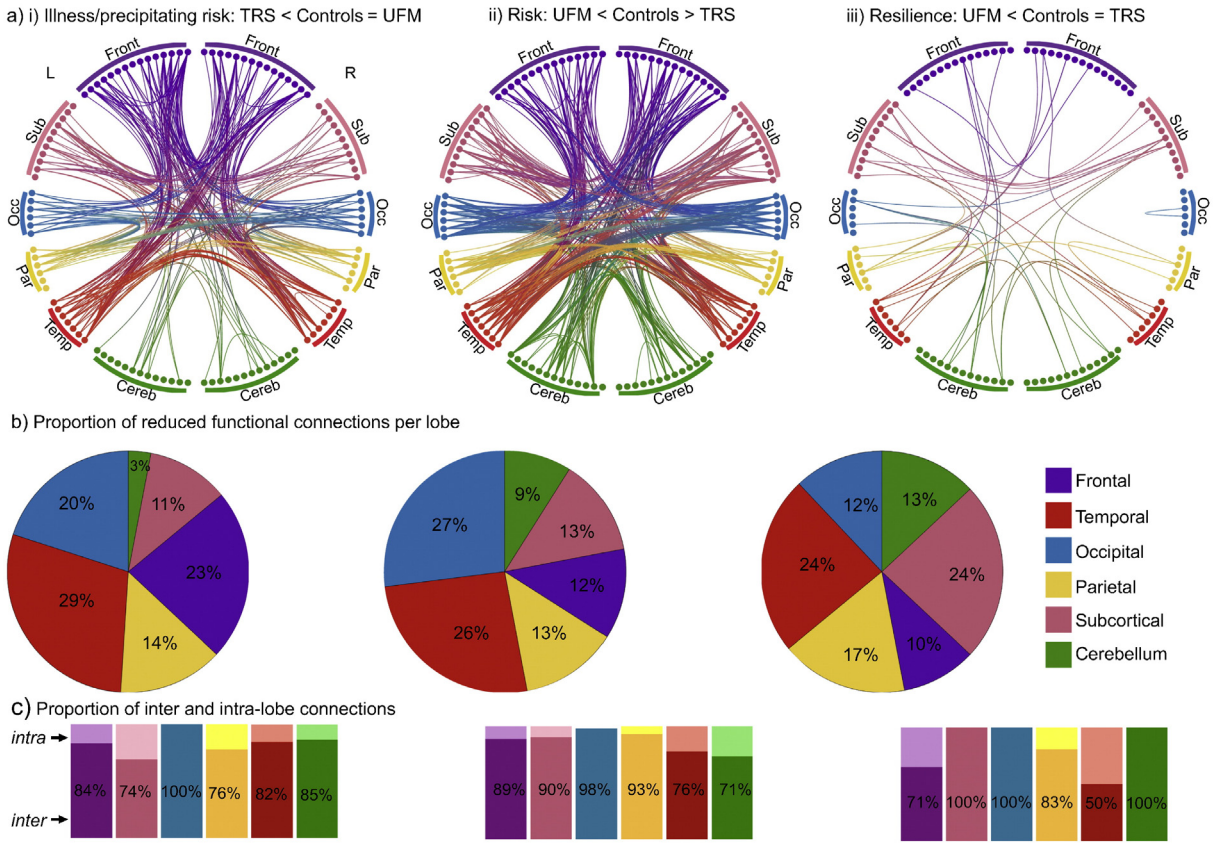

Fig. 2. a) Connectograms for the three most populated quadrants of the two-dimensional risk and resilience space (Fig. 1b): i) risk (TRS < controls; UFM < controls), ii) illness/precipitating risk (TRS < controls; controls = UFM), iii) resilience (UFM < controls; TRS = controls). Connectograms comprise all connections that reside within a given quadrant (open circles in Fig. 1b). Nodes represent brain regions, which are grouped on the connectogram circumference according to lobes. Left hemisphere nodes are shown on the left side of the connectogram. Connections are colored based on the lobe or lobes in which the corresponding pair of nodes resides. b) Normalized proportion of connections comprising each of the six brain lobes for the three connectograms shown in Panel a. Normalized proportions were obtained by dividing the number of connections associated with a lobe by the number of nodes comprising the lobe. c) Proportion of inter-lobar and intra-lobar connections for the three connectograms shown in Panel a. TRS = treatment-resistant schizophrenia; UFM = unaffected family members; L = left hemisphere; R = right hemisphere; Front = frontal; Sub = subcortical; Occ = occipital; Par = parietal; Temp = temporal; Cereb = cerebellum.

the four modules (visual module; $p = 0.041$ and the cerebellar module; $p < 0.001$), with UFM and TRS showing increased local efficiency relative to controls (Fig. 3c). See Fig. 3d for the median local efficiency per weight threshold per module for each group.

3.3. Relationships with clinical symptoms and functioning

No associations between rs-FC and clinical symptoms and functioning were found.

4. Discussion

Investigating functional brain networks in TRS individuals, UFM and unrelated healthy controls enabled us to identify resting-state functional connections and functional network properties that represent putative markers of schizophrenia risk and resilience. TRS and UFM shared numerous rs-FC reductions, and these were operationalized as schizophrenia risk markers. Alterations in rs-FC that were unique to UFM, a marker of resilience, were rarer. Topological analyses indicated that UFM and TRS were more resilient to connectivity disruptions, suggesting that local network efficiency may be a resilience endophenotype for the disorder.

4.1. Functional connections associated with risk and precipitating risk/illness effects

Numerous connections were reduced exclusively in the TRS group, and this is likely representative of prolonged illness duration, medication effects and/or precipitating risk for the disorder. Remarkably, 64% of the reduced connections in TRS were also present in UFM and, as a result, two important questions arise. 1) Are the shared connections indicative of an endophenotype or heritable risk for the disorder? 2) Are the reduced connections that are found only in the TRS group driving factors in the development of schizophrenia?

The reduced functional connections that were classified as risk markers (those found in both TRS and UFM relative to controls) predominantly involved temporal and occipital lobes, suggesting that reduced rs-FC of these regions is not necessarily secondary to the disorder, but instead may be a product of genetic and other heritable risk factors associated with schizophrenia. Given schizophrenia and functional connectivity are both highly heritable (van den Heuvel et al., 2013), this shared dysconnectivity may represent the effects of genetic variants conferring risk for the disorder. Perhaps if dysconnectivity is confined to temporal and occipital regions and does not increase in severity, the disorder may not precipitate. However, if temporal lobe dysconnectivity is increased in conjunction with a relatively large increase in frontal lobe dysconnectivity (as seen in the connections categorised as precipitating risk/illness effects), the risk phenotype may precipitate psychosis onset.

Conversely, the remaining 36% of reduced connections that were found only in the TRS group may play a precipitating or perpetuating role in the divergence between one family member versus another developing the disorder. This reduced frontal and temporal rs-FC could be a driving factor in the development of schizophrenia, however we are unable to determine whether the dysconnectivity preceded the onset of psychosis, or developed as a result of illness progression. In line

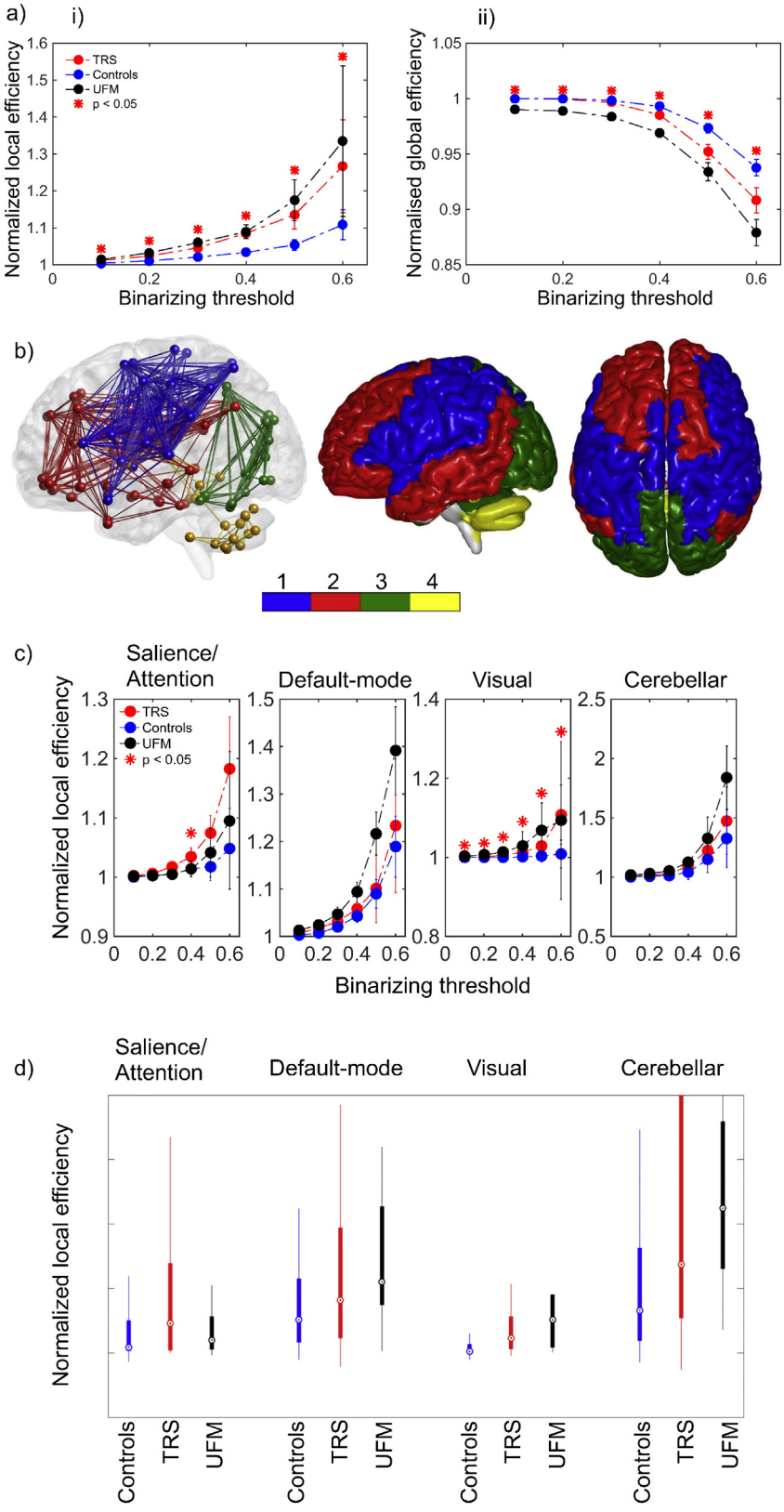

Fig. 3. a) Normalized global and local efficiency plotted as a function of the graph binarizing threshold for TRS (red), UFM (black) and control (blue) groups. Plots represent group medians. Normalized efficiency is the ratio of an individual's measured efficiency to the mean efficiency in an ensemble of degree-preserving random graphs. Error bars denote one standard deviation. Red stars mark significant differences ($p < 0.05$). b) Anatomical representation of the four functional modules: 1) Salience/attention module, 2) Default-mode module, 3) Visual module, 4) Cerebellar module. Efficiency was separately computed for the subnetworks defined by each of these modules. The majority of between-group differences in rs-FC were inter-modular, and thus connection density was better matched across the three groups for these modular sub-networks, thereby alleviating the confound of between-group differences in connection density. c) Normalized local efficiency for each module plotted as a function of the graph binarizing threshold. Plots represent group medians for TRS (red), UFM (black) and controls (blue). The error bars denote one standard deviation. Red stars mark significant differences ($p < 0.05$). d) Area under curve (AUC) of normalized local efficiency for each module and for TRS (red), UFM (black) and control (blue) groups. The central mark (circle) is the median, the edges of the box are the 25th and 75th percentiles and the whiskers extend to the most extreme data points. TRS = treatment-resistant schizophrenia, UFM = unaffected family members.

with our previous study that utilised the same TRS cohort, we did not find evidence of an association between reduced rs-FC and symptomatology (Ganella et al., 2017). This may represent a downstream ceiling effect of brain connectivity on symptom severity due to prolonged illness duration and long-term medication effects. However, given that over half of the reduced connections found in TRS were also present in UFM, it could be questioned as to what the functional implications of this dysconnectivity are for healthy biological relatives. Reduced rs-FC could be contributing to cognitive deficits that are reported as being more prevalent in UFM of individuals with schizophrenia relative to unrelated healthy controls (Snitz et al., 2006), yet our UFM group did not differ from controls in IQ, global or social functioning. This study however did not analyze specific neuropsychological tests, thus, future research should explore the relationship between cognition and rs-FC in UFMs.

4.2. Functional connections associated with resilience

Approximately 5% of the functional connections that differed between groups were classified as resilience markers. These connections were uniquely reduced in UFM, without significant differences between TRS and healthy controls, and predominantly involved temporal and subcortical regions. The present study however was cross-sectional, and therefore, could not explicitly test for evidence of resilience against the development of schizophrenia. Although our findings cannot be interpreted beyond a classification of connections that are significantly different between TRS, UFM and the general population, we propose three alternative explanations for the unique alterations in rs-FC seen in UFM:

1. Reduced rs-FC predominantly involving temporal and subcortical regions may be an expression of susceptibility genes for schizophrenia, however given the reduction is much less widespread and in different anatomical locations, the disorder may not emerge. Additionally, the fact that the frontal lobe was least implicated may indicate that UFM do not possess the necessary pathophysiology to precipitate onset of schizophrenia.
2. The TRS group could have once displayed this same pattern of dysconnectivity, however antipsychotic treatment over time restored the reduced rs-FC to normal levels seen in controls, whereas UFM who have never been prescribed antipsychotics still show reduced rs-FC in these regions. This theory is supported by evidence of antipsychotic treatment normalizing abnormal rs-FC in schizophrenia patients over time almost to levels observed in controls (Li et al., 2016; Sarpal et al., 2015).
3. If the genesis of the disorder is in temporal regions, additional reduced temporal rs-FC in UFM may be a protective mechanism to constrain or isolate pathology to the temporal lobes. Studies have shown reduced temporal cortical thickness (Goghari et al., 2007; Prasad et al., 2010) and volume (McIntosh et al., 2011) in UFM relative to controls. By reducing temporal rs-FC, the amount of communication the temporal lobes have with other brain areas may be restricted and consequently, the neural impairment might be limited. It could be that during neurodevelopment, genetic and/or environmental influences impeded this protective strategy in TRS individuals, leading to widespread dysconnectivity and the emergence of schizophrenia.

4.3. Network efficiency

We found higher local and reduced global efficiency in UFM and TRS relative to controls. Previous work by Lo et al. (2015), also reported increased local efficiency in UFM, however, in contrast to the present findings, they found higher local efficiency in the order schizophrenia patients > UFM > controls. One potentially confounding factor of the methodology used by Lo and colleagues was that they investigated efficiency as a function of network density, which can bias group comparisons if groups have different overall rs-FC strength (Fornito et al., 2016). In an attempt to circumvent this limitation, the current study used connection weight thresholding, otherwise known as absolute thresholding. Higher local efficiency implies more efficient local computation and increased resilience to the failure of individual nodes. Therefore, this increased robustness to random attack in UFM may represent a form of resilience. Here, we debate the possibility that increased local efficiency is a protective mechanism, however, one might question why we also see the greatest decrease in global efficiency in UFM, which generally implies poorer global integration and communication within a network. Small-worldness is a balance between integration (global efficiency) and segregation (local efficiency). Tipping the balance towards one side, in this case, towards local efficiency, may be at the expense of a reduction in global efficiency. Perhaps the most important protective factor is increased local efficiency, despite a consequential decrease in global efficiency, that is, the benefit of increased resilience outweighs the disadvantage of reduced global integration.

In order to further explore this increase in local efficiency, we performed modular decomposition, partitioning the brain into four modules and investigated group differences in local efficiency within modules. We found higher local efficiency in TRS and UFM relative to controls in all four modules, and this was significant for the visual and the cerebellar modules. This suggests that the loss of long-range connections in TRS and UFM individuals is an unlikely explanation for the increase in local efficiency.

4.4. Limitations

A limitation of this study was the relatively small UFM sample. However, a study of a phenotypically homogeneous patient cohort is an important advantage that maximizes the likelihood of reproducibility despite a small sample size, as demonstrated with replication of our findings with an alternative parcellation atlas. Another important limitation is the difficulty in disentangling precipitating risk from the effects of illness duration and/or prolonged antipsychotic use, both of which have been linked to structural (Navari and Dazzan, 2009) and functional changes in brain connectivity (Y. Liu et al., 2008; Sarpal et al., 2015). Further, we did not have information pertaining to antipsychotic medication adherence in the TRS group, and non-adherence can play a large role in non-response to treatment. Therefore, future studies should document and control for treatment compliance to ensure a consistent and reliable classification of TRS. Medication effects however cannot fully explain our results, as similar profiles were evident in UFM who were mostly un-mediated, with only two participants taking antidepressants at the time of scanning (analyses were re-run excluding these two participants and results remained significant, see Supplementary Material).

5. Conclusions

TRS individuals and UFM share widespread rs-FC deficits, which predominantly involve temporal and occipital regions and represent a putative risk endophenotype. Deficits that are unique to TRS involve frontal and temporal regions, and represent risk for the disorder and/or the effect of prolonged illness. Rs-FC alterations that are unique to UFM are rarer and mostly involve temporal and subcortical regions. Together with increased local network efficiency, these UFM-specific characteristics may confer resilience to schizophrenia, despite familial risk for the disorder. In view of the aforementioned limitations, particularly the small UFM sample size, the findings must be considered as preliminary. Future work with larger cohorts will further our understanding of putative genetic vulnerability markers that will improve the identification of endophenotypes of TRS, and in turn streamline treatment approaches with increased efficiency and specificity in diagnosis and therapeutic approaches.

Contributors

Author Zalesky designed the functional connectivity protocol and was imperative to the methodology and analysis of the neuroimaging data. Author Seguin assisted in the design and execution of the graph theory analyses. Author Pantelis, author Phassouliotis and author Everall were imperative to the design, recruitment and execution of the study. Author Whittle, author Bousman and author Bartholomeusz assisted in the statistical design of the study. Author Di Biase and author Wannan further assisted the statistical design of the study, recruitment of participants and management of functional resting-state data. Author Ganella performed all the neuroimaging and statistical analyses and wrote the manuscript. All authors contributed to and have approved the final manuscript.

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Conflicts of interest

The Authors have declared that there are no conflicts of interest in relation to the subject of this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2017.07.014.

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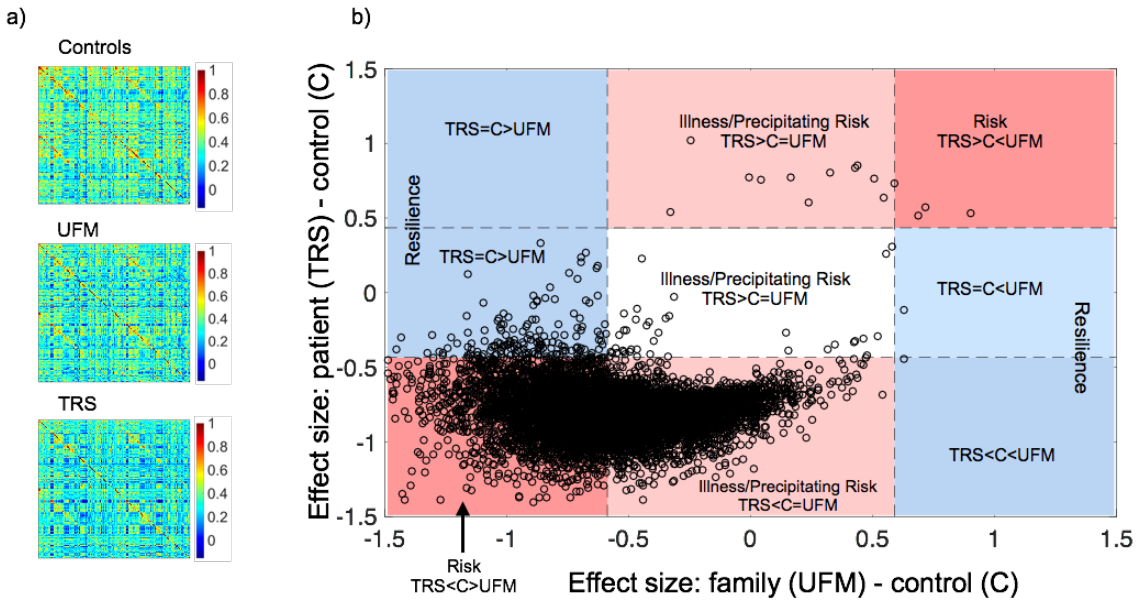
Supplementary Material

Figure 1.



Glasser parcellation atlas verification.

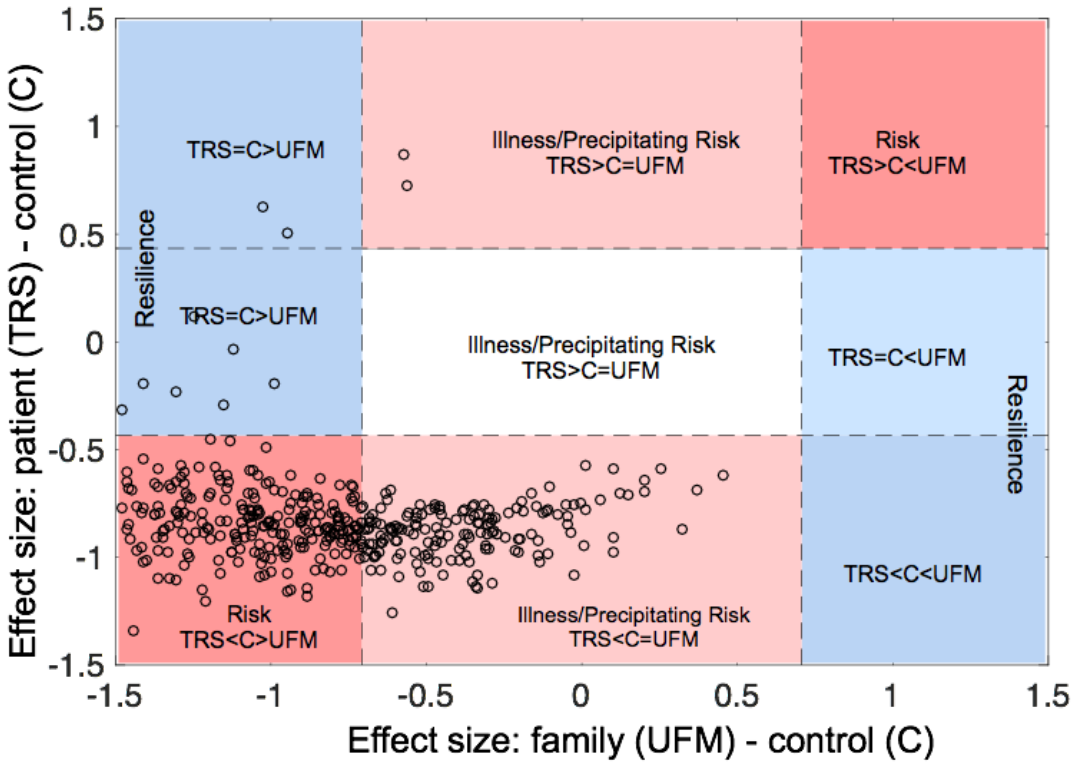
a) Group-averaged 360 × 360 rs-FC matrices for control, UFM and TRS groups. Connectivity matrix elements contain r-to-z transformed, group-averaged correlation coefficients for 64,620 pairs of regions. Matrix elements in the upper sub-block contain left hemisphere connections, those in the lower sub-block represent the right hemisphere and the off-diagonal sub-block contains inter-hemispheric connections. The prominent off-diagonal elements correspond to contralateral homologous regions.

b) Two-dimensional representation of the risk and resilience classification scheme. Each open circle represents a connection for which the null hypothesis of equality in mean rs-FC between TRS, UFM and control groups was rejected ($p<0.05$, familywise error corrected for 64,620 tests). The horizontal axis is the effect size for post-hoc t-tests comparing UFM with healthy controls and the two dashed vertical lines correspond to $\alpha = 0.05$ cut-off thresholds for the t-test. Analogously, the vertical axis and two horizontal dashed lines relate to post-hoc t-tests comparing TRS and healthy controls. The two-dimensional space is subdivided into 9

quadrants, with the central quadrant (white) representing cases where both post-hoc t-tests were not significant. At least one of the post-hoc t-tests was significant for the other 8 quadrants. The significance and direction of the post-hoc t-tests determined whether connections were assigned to a quadrant associated with i) risk (red quadrants), ii) resilience (blue quadrants), or iii) illness/precipitating risk (pink quadrants).

TRS = Treatment-resistant schizophrenia; UFM = Unaffected family member; C = Controls

Figure 2.



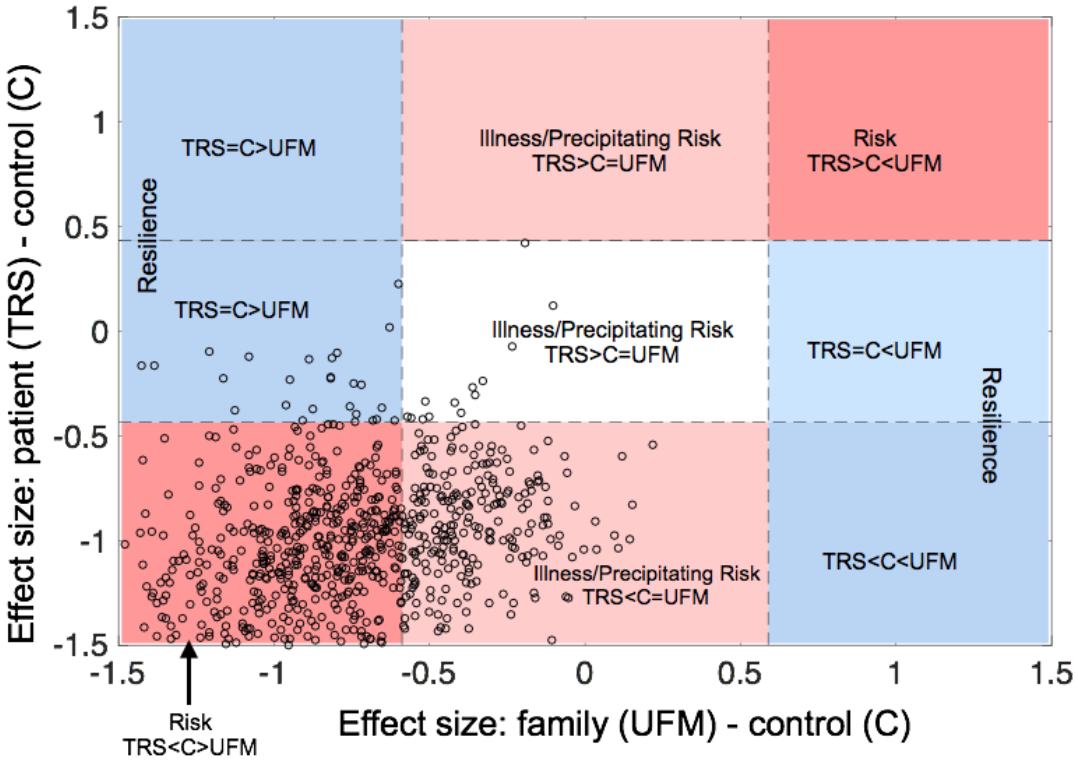
Sensitivity analysis using the AAL parcellation atlas excluding UFM (n = 6) who did not have an affected family member included in this study.

Two-dimensional representation of the risk and resilience classification scheme. Each open circle represents a connection for which the null hypothesis of equality in mean rs-FC between TRS, UFM and control groups was rejected ($p<0.05$, familywise error corrected for 6,670 tests). The horizontal axis is the effect size for post-hoc t-tests comparing UFM with healthy controls and the two dashed vertical lines correspond to $\alpha = 0.05$ cut-off thresholds for the t-test. Analogously, the vertical axis and two horizontal dashed lines relate to post-hoc t-tests comparing TRS and healthy controls. The two-dimensional space is subdivided into 9 quadrants, with the central quadrant (white) representing cases where both post-hoc t-tests were not significant. At least one of the post-hoc t-tests was significant for the other 8 quadrants. The significance and direction of the post-hoc t-tests determined whether

connections were assigned to a quadrant associated with i) risk (red quadrants), ii) resilience (blue quadrants), or iii) illness/precipitating risk (pink quadrants).

TRS = Treatment-resistant schizophrenia; UFM = Unaffected family member; C = Controls

Figure 3.



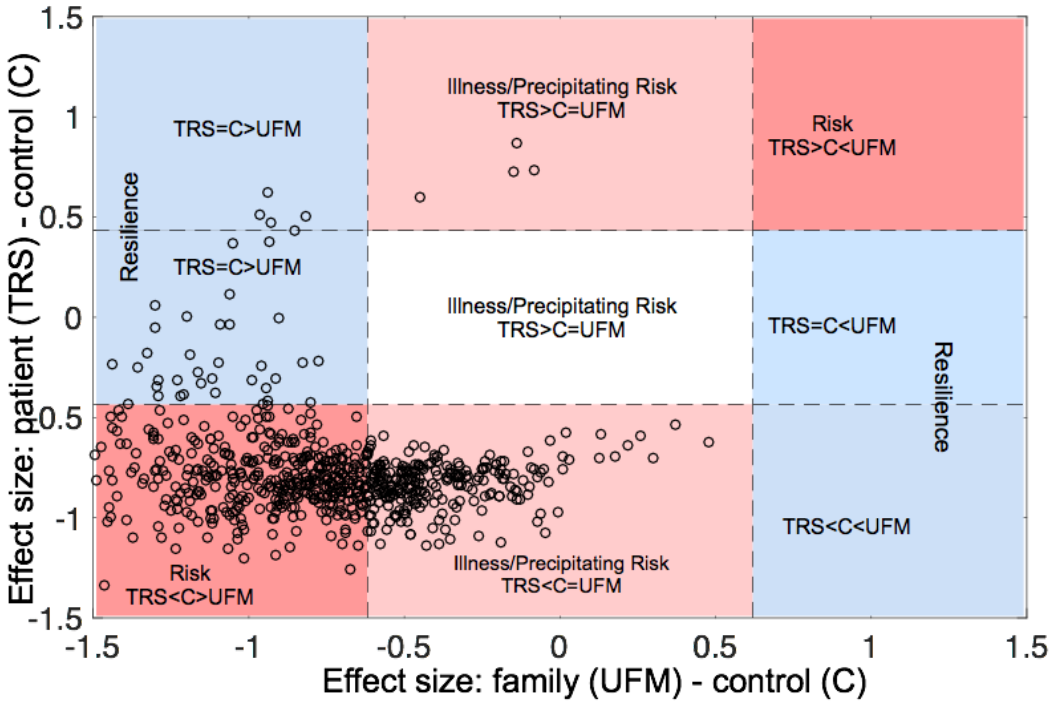
Reproduction of Figure 1b after excluding all males, UFM males (n = 2), TRS males (n = 30), control males (n = 30) using the AAL parcellation atlas.

Two-dimensional representation of the risk and resilience classification scheme. Each open circle represents a connection for which the null hypothesis of equality in mean rs-FC between TRS, UFM and control groups was rejected ($p<0.05$, familywise error corrected for 6,670 tests). The horizontal axis is the effect size for post-hoc t-tests comparing UFM with healthy controls and the two dashed vertical lines correspond to $\alpha = 0.05$ cut-off thresholds for the t-test. Analogously, the vertical axis and two horizontal dashed lines relate to post-hoc t-tests comparing TRS and healthy controls. The two-dimensional space is subdivided into 9 quadrants, with the central quadrant (white) representing cases where both post-hoc t-tests were not significant. At least one of the post-hoc t-tests was significant for the other 8 quadrants. The significance and direction of the post-hoc t-tests determined whether

connections were assigned to a quadrant associated with i) risk (red quadrants), ii) resilience (blue quadrants), or iii) illness/precipitating risk (pink quadrants).

TRS = Treatment-resistant schizophrenia; UFM = Unaffected family member; C = Controls

Figure 4.



Reproduction of Figure 1b after excluding all UFM participants on antidepressant medication (n = 2) using the AAL parcellation atlas.

Two-dimensional representation of the risk and resilience classification scheme. Each open circle represents a connection for which the null hypothesis of equality in mean rs-FC between TRS, UFM and control groups was rejected ($p<0.05$, familywise error corrected for 6,670 tests). The horizontal axis is the effect size for post-hoc t-tests comparing UFM with healthy controls and the two dashed vertical lines correspond to $\alpha = 0.05$ cut-off thresholds for the t-test. Analogously, the vertical axis and two horizontal dashed lines relate to post-hoc t-tests comparing TRS and healthy controls. The two-dimensional space is subdivided into 9 quadrants, with the central quadrant (white) representing cases where both post-hoc t-tests

were not significant. At least one of the post-hoc t-tests was significant for the other 8 quadrants. The significance and direction of the post-hoc t-tests determined whether connections were assigned to a quadrant associated with i) risk (red quadrants), ii) resilience (blue quadrants), or iii) illness/precipitating risk (pink quadrants).

TRS = Treatment-resistant schizophrenia; UFM = Unaffected family member; C = Controls

Further information on graph theoretical methods used

Network efficiency

Global and local network efficiency were computed and compared between all three groups. Global efficiency quantifies the capacity for a network to support concurrent communication between multiple brain regions (Bullmore & Sporns, 2012). In contrast, local efficiency characterizes how well communication is supported within a local neighborhood of regions, and can be interpreted as a measure of network resilience to a single regional disruption (Rubinov & Sporns, 2010; Wang, Zuo, & He, 2010). More specifically, global efficiency is the reciprocal of shortest path length, averaged over all pairs of regions (Fornito, 2015). Local efficiency is the same quantity computed for the set of neighbors of a given region and then averaged over all regions(Fornito, 2015).

Each subject's functional network was transformed into a binary graph by eliminating connections with FC below a fixed threshold, otherwise known as weight-based thresholding (Fornito, 2015). A range of FC thresholds between 0.1 and 0.6 were considered. Local and global efficiency were computed for each threshold and normalized with respect to 100 degree-matched random networks generated with the Maslov-Sneppen rewiring algorithm (Milo et al., 2002). Normalization was performed to control for potential differences in connection density between individuals. These normalized measures of efficiency were then compared between the three groups at each threshold and the area-under-curve (AUC) was used to provide a summary measure for each participant.

Module Delineation

To further characterize brain network topology, and ensure our normalization process was not driving results, we investigated the modular organization of brain networks and the local efficiency within each module. A module generally refers to a subset of nodes which are more densely connected to other nodes in the same module than to nodes outside the module

(Radicchi, Castellano, Cecconi, Loreto, & Parisi, 2004). The modular decomposition of the network was identified using the Fast Louvain's Algorithm (Blondel, Guillaume, Lambiotte, & Lefebvre, 2008). This initially involves each node of the network being assigned a different module, so that initially, the number of modules is equal to the number of nodes (n=116). Following this, the algorithm consists of two stages that are repeated iteratively. First, for each node, the algorithm considers each of its neighbors (edges) and computes the gain of modularity that would be obtained if the node was placed in the same module as its neighbor. A node may leave its assigned module and join a different module which is in its direct neighborhood, but only if this change leads to an increase in modularity (Blondel et al., 2008). This process is applied sequentially, cycling through every node until no individual move can improve the modularity, at which point the first stage will stop. The second stage involves building a new network whose nodes are partitioned into the modules identified in the first stage. This generates a set of modules that have high intra-modular connectivity and relatively sparse inter-modular connectivity. For details of this algorithm see (Newman & Girvan, 2004).

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CHAPTER 5:

General discussion

5.1. Thesis overview and summary of results

In this thesis, we explored resting-state functional brain connectivity and network topology in early and late stage schizophrenia, as well as in a group of unaffected first-degree relatives of individuals with schizophrenia, to elucidate what effect psychosis has on functional networks at different illness stages. Firstly, to explore the early stage of the psychosis continuum, we investigated whole-brain resting-state functional connectivity (rs-FC) and network topology in a first-episode psychosis (FEP) sample at baseline, and at 12-months follow-up. This group was a conventional FEP group in that participants were within the first two years of being diagnosed with a schizophrenia-spectrum disorder and were aged 15-25 years. Secondly, to determine the neurobiological differences/similarities between early and late schizophrenia groups, we investigated rs-FC and topology in a chronic, late phase treatment-resistant schizophrenia (TRS) cohort. This group represents the other end of the continuum, a severely ill population that has had the disorder for typically over a decade. Thirdly, to address the last aim of this thesis, we explored whole-brain rs-FC and topology in unaffected family members (UFM) to determine whether they shared any network abnormalities that were evident in probands (the TRS group). This enabled us to investigate whether UFM demonstrated any rs-FC or topological markers of resilience that may have been a protective factor in preventing them from developing the disorder.

In *Chapter 2*, we reported that FEP individuals did not show any significant differences in rs-FC or functional network topology relative to healthy controls. This result of no difference was evident at baseline, and at 12-months follow-up. Additionally, the FEP group did not show any abnormal change in rs-FC or topology over 12-months relative to that of normal aging observed in the healthy controls. These findings are contrary to the hypotheses and suggest that our FEP group appear to be unaffected in terms of rs-FC network disturbances

during this early illness stage, and that this may reflect a good clinical prognosis. In contrast to the lack of rs-FC and topological differences observed in the FEP cohort, in *Chapter 3*, we reported widespread reduced rs-FC in the TRS group relative to healthy controls, predominantly involving temporal, occipital and frontal lobes. Topologically, TRS patients showed increased local and decreased global efficiency relative to healthy controls. Relatedly, in *Chapter 4*, we found reduced rs-FC to also be evident in the UFM of TRS patients relative to healthy controls, albeit to a lesser severity than that observed in the TRS group relative to controls. TRS and UFM shared frontal and occipital rs-FC deficits, which were hypothesised to represent a 'risk' endophenotype. TRS patients also showed additional reductions in frontal and temporal rs-FC, and we speculated that these deficits that were specific to the TRS group may be due to risk that precipitated psychosis in vulnerable individuals, and/or illness-related effects. Topologically, functional brain networks were found to be reduced in global efficiency and more topologically resilient (i.e. increased local efficiency) in UFM and TRS patients relative to healthy controls, and these findings were most pronounced in the UFM group. Overall, findings support the notion that the integrity of rs-FC networks differs across the psychosis spectrum (Pantelis et al., 2005).

5.2. General discussion

5.2.1. Rs-FC abnormalities in early and late psychosis

An interesting observation that emerged from the thesis is that our FEP cohort showed no observable network deficits and no abnormal change in network measures over 12-months relative to healthy controls. This result of no difference in functional brain network integrity in FEP was unexpected and inconsistent with our hypotheses, particularly given the majority of fMRI research conducted in FEP populations have reported numerous rs-FC abnormalities relative to healthy controls.

There is much evidence to support the notion that schizophrenia-spectrum disorders develop over time, and that their trajectory can be described in at least three stages; the prodrome, the FEP, and chronic phase (Agius, Goh, Ulhaq, & McGorry, 2010; Singh et al., 2005). It is also known that not every individual who experiences a first-episode of psychosis will go on to develop a chronic schizophrenia-spectrum disorder, as up to 60% will not experience a second psychotic episode (this figure decreases upon discontinuation of medication) (Menezes, Arenovich, & Zipursky, 2006). Our finding of no observable network impairment in FEP could suggest that this sample in particular may have a good prognosis, with fewer associated neurobiological deficits. As discussed in *Chapter 2*, this may be due to a number of factors, such as the efficacy of early intervention and pharmacological therapy. It could be that treatment, such as cognitive behavioral therapy (CBT) in conjunction with antipsychotic medication has positively impacted on the brain during this critical period of neurodevelopment. There is growing evidence that early interventions such as CBT can produce lasting network changes in a range of disorders (Barsaglini, Sartori, Benetti, Pettersson-Yeo, & Mechelli, 2014). Supporting this notion, previous work has found that CBT can normalise abnormal functional connectivity during a facial processing task in psychosis patients (Mason, Peters, Dima, Williams, & Kumari, 2016), and in one study, changes in brain connectivity following CBT predicted long-term recovery in schizophrenia patients (Mason, Peters, Williams, & Kumari, 2017). Furthermore, a few studies have shown that some, but not all rs-FC abnormalities that are present during the early illness phase of psychosis normalise after initial treatment with antipsychotic medication (Abbott, Jaramillo, Wilcox, & Hamilton, 2013; F. Li et al., 2016; Lui et al., 2010; Sambataro et al., 2010; Sarpal et al., 2015). Therefore, perhaps a combination of these therapeutic approaches has contributed to preventing or delaying rs-FC abnormalities developing in our FEP cohort.

With this said, it is also possible that a subset of these FEP participants might demonstrate abnormal rs-FC and network topology relative to controls, and between-group differences may have been diluted by those who do not show any network abnormalities. Characterising treatment factors that predict positive neurodevelopmental pathways is an important step forward in improving future early psychosis intervention strategies; thus, future research into why our particular cohort did not display any rs-FC or topological deficits is necessary.

Additionally, we did not see any abnormal change in rs-FC over time in FEP. It could be that 12-months is an insufficient follow-up period to identify significant changes in rs-FC and network topology that are associated with early stage psychosis. Perhaps a longer duration of time is required to truly recognise neural abnormalities that become progressively evident in FEP patients as they move from this early illness stage into later more severe illness stages, diverging from the patients with a good prognosis that only experience an initial FEP. While rs-FC and network topology have been shown to change over 3 years in healthy adults (Fjell et al., 2017), there is little evidence to show that it changes significantly over a 12-month period in the healthy population. In the present thesis, we found no main effect of time on any network measure investigated in FEP or healthy controls. Therefore, perhaps longer follow-up periods are necessary to detect significant brain changes that are occurring in any population, healthy or FEP. Although not in relation to rs-FC, in line with the current findings, it has been shown in a previous study that FEP individuals did not experience structural brain changes over a 12-month period compared with healthy controls (Haukvik et al., 2016). Conversely, another study did show a significant change in structural brain morphology in FEP over a 3-year follow period (Ayesa-Arriola et al., 2013), perhaps suggesting that our FEP sample may display significant functional neurobiological changes if they are followed-up at 2-3 years (Bartholomeusz et al., 2017; Sun et al., 2009). Although we

did not observe any rs-FC abnormalities in FEP individuals, a separate study utilising the same FEP and healthy control cohorts did report mild white matter disruptions circumscribed to the anterior fibers of the corpus callosum (Di Biase et al., 2017). This suggests that deficits in anatomical connectivity precede deficits in functional connectivity (if deficits in functional connectivity ever do eventuate in this cohort), and further highlights the need for longitudinal studies in FEP cohorts with longer follow-up periods to extensively characterise the stages and order in which neurobiological abnormalities emerge during early psychosis.

When interpreting the present findings in terms of the clinical staging model, the current FEP results did not reveal any pathological brain abnormalities/changes that could be used to characterise the FEP clinical stage, but they did reveal a number of network abnormalities that could characterise the later more severe illness stage. The clinical staging model recognises the need to combine dimensional and categorical models to fully encompass a broader range of clinical and biological phenotypes and in turn, encompass the full spectrum of the disorder (Agius et al., 2010). Our finding of no neurobiological abnormalities in FEP may represent an extended phenotype of the disorder that may be more resilient to neuropathology, and is more continuous with the healthy population (McGorry, 2014). As previously discussed in *Chapter 2*, the lack of abnormal rs-FC observed in this sample was mirrored by a relatively mild to moderately ill clinical presentation relative to other studied FEP cohorts. This relationship between relatively mild clinical phenomena and 'normal' neural networks further supports the differentiation between early, less severe clinical stages, from established forms of the disorder, and may suggest that this FEP group will not progress to experience a chronic form of schizophrenia. In saying this, the FEP individuals did have clinical symptoms, which may precede and potentially cause later neurobiological changes. Further understanding the relationship between biological phenotypes and stage of disorder

will help to elucidate the boundaries that distinguish core biological processes from associated illness effects, and this will progress our understanding of the etiology of schizophrenia-spectrum disorders (Bartholomeusz et al., 2017; McGorry et al., 2007; Wood, Yung, McGorry, & Pantelis, 2011).

In contrast to the null results reported in FEP patients in *Chapter 2*, *Chapter 3* showed widespread reduced rs-FC in TRS patients relative to controls, predominantly involving frontal (particularly the paracentral lobule), temporal (particularly the fusiform and Heschl's gyri) and occipital (particularly the cuneus) brain regions. In terms of the clinical staging model, this group of patients sit on the chronic end of the continuum, and represent the most established and severe form of schizophrenia. Therefore, it is somewhat unsurprising that the neuropathology in this cohort will differ greatly from that of the FEP group explored in *Chapter 2*. A key concept of the clinical staging model in psychosis is that early and late clinical stages can be differentiated based on both clinical presentation, including illness duration/chronicity as well as biological factors, and the current results support this notion (i.e., neurobiological changes were apparent in TRS but not FEP patients). Although the pathophysiology of reduced functional connectivity is not fully understood, one theory suggests that disrupted anatomical connectivity, as reported by diffusion tensor-imaging (DTI) studies (Kubicki et al., 2007), may underlie disturbances in the temporal co-activation of brain regions. Widespread white matter pathology in the form of reduce fractional anisotropy has previously been reported in a large chronic schizophrenia cohort (n=326), suggesting a more global impact of schizophrenia on connectivity in the more severe cases of the disorder, which is in line with the present findings (Klauser et al., 2017). Further, progressive grey matter loss that is a characteristic feature of psychotic illness may also play a role in the functioning of these regions and the functional connectivity between them. It

would be of interest to investigate these properties in our FEP and TRS cohorts to determine whether a decline in grey matter accompanies the functional connectivity deficits observed here. Future research using a combination of modalities including structural-MRI, DTI in addition to BOLD-fMRI would further determine the anatomical underpinnings of the 'dysconnectivity' observed in schizophrenia, and the timing and stage in which these abnormalities occur.

The temporal lobes have been consistently implicated in the pathophysiology and symptomatology of schizophrenia, including the transition to psychosis (Pantelis et al., 2003; Takahashi et al., 2009; Takahashi, Wood, et al., 2010), with findings of volumetric reductions (Kuroki et al., 2006), hypoactivation (Takei et al., 2013) and disrupted structural (Minami et al., 2003) and functional connectivity (Meyer-Lindenberg et al., 2001), and the current findings of impaired temporal lobe functional connectivity in a TRS group adds to this existing literature. The fusiform gyrus is known to play a central role in the recognition of words, colour and in particular facial information (Gauthier, Tarr, Anderson, Skudlarski, & Gore, 1999) and the Heschl's gyrus (a region of the primary auditory cortex) is understood to be especially involved in auditory hallucinations (Dierks et al., 1999). Although neither the fusiform or Heschl's gyrus have been specifically investigated in a TRS population, previous studies have found both regions to show volumetric reductions in first-episode schizophrenia (Hirayasu et al., 2000; C. U. Lee et al., 2002; Takahashi, Yucel, et al., 2010), chronic schizophrenia (Hirayasu et al., 2000; Kwon et al., 1999; Onitsuka et al., 2003) as well as reduced fusiform functional activation during facial emotion processing (Johnston, Stojanov, Devir, & Schall, 2005) and both increased as well as decreased Heschl's gyrus functional activation and connectivity during auditory hallucinations in chronic schizophrenia groups (Shinn, Baker, Cohen, & Ongur, 2013).

Further, the observed reduced temporo-occipital (in particular the cuneus) rs-FC in the TRS group may represent underlying biological disturbances that could be precipitating or exacerbating visual hallucinations. Reduced fractional anisotropy in white matter tracts connecting temporal and occipital regions (Bora et al., 2011; Ellison-Wright & Bullmore, 2009; Klauser et al., 2017) have been associated with visual hallucinations in schizophrenia and additionally, disturbances in white matter and coticocortical connectivity involving the cuneus (Moran et al., 2015) and temporo-occipital areas are seen in children with schizophrenia, most of whom have a severe treatment-resistant phenotype and are treated with clozapine (Zalesky et al., 2015). Further to this, longitudinal fronto-occipital white matter volume reductions have been observed in at-risk individuals who transitioned to psychosis (Walterfang et al., 2008). It has been hypothesized that a decrease in structural and/or functional temporo-occipital and fronto-occipital connectivity may lead to modulatory control deficits, resulting in hyper-excitability of brain regions and the subsequent emergence of visual hallucinations (Carter & Ffytche, 2015). Thus, the reduced neural synchrony between temporo-occipital and fronto-occipital regions may have downstream modulatory effects of reducing visual processing accuracy, which could in turn lead to perceptual disturbances such as visual hallucinations. This interpretation, however, is speculative, as only 7 out of the 42 TRS patients were reported as experiencing visual hallucinations at the time of assessment, and we found no relationship between occipital rs-FC and positive symptomatology. Therefore, although disruptions to occipital rs-FC might underlie visual hallucinations, it is unlikely that the small fraction of participants experiencing these symptoms is driving the results. However, this is a complex issue, as patients often do not report visual hallucinations. Additionally, they may have had them in the past, but not at the time of assessment, hence, it is unlikely that we have the complete clinical picture. Future

studies with greater numbers of patients identified as experiencing visual hallucinations should endeavor to investigate this relationship.

As previously discussed in *Chapter 3*, a theory that has gained recent momentum suggests that TRS may in fact be more accurately understood and interpreted as a distinct subtype of schizophrenia, as opposed to residing on the continuum and representing the most chronic illness phase of the disorder (Farooq, Agid, Foussias, & Remington, 2013; J. Lee et al., 2015). This notion has been supported by recent research findings of dopamine levels appearing normal in the TRS brain (whereas dopamine levels are often reported as abnormal in other schizophrenia cohorts), however glutamate levels are elevated (Demjaha et al., 2014; Demjaha, Murray, McGuire, Kapur, & Howes, 2012). Further to this, most individuals with schizophrenia will show a clinical response to antipsychotic treatment that is correlated with dopamine receptor D2 occupancy (Nord & Farde, 2011). However, TRS patients show little to no clinical response to treatment even when their D2 receptor occupancy is above the therapeutic threshold (Wolkin et al., 1989). This suggests that the dopamine hypothesis may not best explain the neurochemical model of schizophrenia in TRS, and that the disorder may instead be driven by non-dopaminergic abnormalities, such as the glutamate system (Demjaha et al., 2014). As previously discussed, clozapine is an antipsychotic that can be extremely effective in treating TRS, despite having relatively low D2 receptor occupancy (Gillespie, Samanaite, Mill, Egerton, & MacCabe, 2017). One possible mechanism underlying its efficacy may relate to its' ability to attenuate glutamate release, as seen in animal studies (Lopez-Gil et al., 2007). Very little fMRI research has been conducted in TRS populations, limiting the amount of studies with which we can compare to the current findings. However, those that have investigated TRS rs-FC generated mixed results, with one study reporting reduced rs-FC involving temporal regions (Vercammen, Knegtering, den

Boer, Liemburg, & Aleman, 2010) while a later study found increased rs-FC between the bilateral temporal regions and decreased rs-FC between other brain regions (C. U. Lee et al., 2002). These findings in conjunction with the current results of widespread reduced rs-FC do not necessarily point towards specific predictive markers of TRS, but may represent a step towards identifying the neurobiological mechanisms that are underlying resistance to antipsychotic treatment. To further disentangle whether TRS is a distinct subtype or a severe stage of schizophrenia, future research should aim to investigate TRS in comparison to treatment-responsive schizophrenia patients. In addition to both replicating and extending the limited neuroimaging findings, further work is necessary that investigates neurochemical differences between TRS and treatment-responsive patients. This could in turn characterise TRS specific markers that might be causing or perpetuating resistance to available antipsychotic medications, and subsequently inform different approaches to pharmacological intervention.

5.2.2. Rs-FC abnormalities associated with schizophrenia are in part, heritable

Although we were unable to compare network connectivity and topology between TRS patients and treatment-responsive schizophrenia patients, we did extend the classic case control design by investigating network measures in UFM of individuals with TRS. This enabled us to disentangle core genetically driven biological processes/shared environmental effects from epiphenomena and sequelae. We found ~900 functional connections to show significant between-group differences in rs-FC between healthy controls, UFM and TRS patients, and these connections were operationalised as conferring: i) risk for the disorder (connections that were reduced in both UFM and TRS groups relative to controls), ii) precipitating risk/illness effects (connections specific to the TRS group relative to controls and UFM), or iii) resilience to the disorder (connections that were only present (or absent) in

the UFM group, separating them from both TRS and controls).

Interestingly, 61% of connections were found to be reduced in rs-FC strength in both TRS patients and UFM. We hypothesise that these connections may represent a heritable rs-FC marker of familial vulnerability to schizophrenia. Although we did hypothesise that there would be some rs-FC abnormalities in UFM relative to controls, we did not expect to see such a significant overlap in reduced rs-FC between the TRS patients and UFM. This result is especially interesting when it is viewed in light of our findings of no significant difference in rs-FC in FEP individuals relative to healthy controls. Although the UFM group were significantly older than the FEP group, it is surprising that a psychiatrically well UFM group shared over 61% of the impaired rs-FC observed in probands relative to healthy controls, whereas the individuals with a FEP diagnosis (those in *Chapter 2*) show no observable impairments relative to controls. This finding supports the heritability of rs-FC abnormalities associated with psychosis, and to a certain extent, the neurodevelopmental hypothesis of schizophrenia.

The neurodevelopmental hypothesis of schizophrenia was proposed 30 years ago (Murray & Lewis, 1987; Weinberger, 1987), and suggests that the etiology of schizophrenia involves environmental and genetic pathologic processes that occur early during neurodevelopment (Rapoport, Addington, Frangou, & Psych, 2005). Neurodevelopmental abnormalities may occur in utero (Brown et al., 2004), or during childhood through to adolescence and young adulthood (Fatemi & Folsom, 2009; Pantelis et al., 2005). Our finding of shared neural abnormalities in TRS probands and UFM further support the genetic influence on the development of the disorder. Unlike single-gene disorders that have homogenous genetic etiologies such as Huntington disease, schizophrenia is a polygenic disorder that has a

heterogeneous etiology, likely deriving from interactions between several genes and various environmental insults (European Network of National Networks studying Gene-Environment Interactions in et al., 2014; Fatemi & Folsom, 2009). The "2-hit" hypothesis works within the framework of the neurodevelopmental theory in which maldevelopment during 2 critical time points (generally suggested to be in utero or during adolescence) combine to precipitate the onset of schizophrenia (European Network of National Networks studying Gene-Environment Interactions in et al., 2014; Keshavan, 1999). The 2-hit model may explain in part why UFM shared many of the neural deficits observed in TRS patients, yet did not develop the disorder. In line with this model, it is possible that genetic influences in utero may have resulted in the reduced rs-FC observed in TRS patients and UFM, and perhaps, this was the heritable "first-hit". Following this, environmental factors such as obstetric or perinatal complications (i.e. hypoxia or viral infections), or trauma such as sexual abuse may have caused a "second-hit" (Keshavan & Paus, 2015), resulting in further neuropathology such as the reduced rs-FC observed in the TRS group alone (precipitating risk/illness effects). The experience of this "second-hit" may be the difference between one family member developing the disorder, versus another not. Future research investigating the influence of environmental factors on connectivity in TRS and UFM is needed to corroborate this speculation. It is possible that the 34% of reduced connections that were found only in the TRS group may have been a driving factor in the development of the disorder, however, it is impossible to determine whether this dysconnectivity preceded the onset of schizophrenia, or developed as a result of illness progression and medication effects. Therefore, we cannot accurately differentiate between what are precipitating risk and what are illness effects in this case.

Interestingly, the majority of 'risk' connections found in TRS patients and UFM

predominantly involved temporal and occipital brain regions, the same two lobes where we observed widespread reduced rs-FC in the TRS group relative to controls (*Chapter 3*). This suggests that reduced rs-FC concerning these regions is not solely secondary to the disorder, but instead may be in part a product of heritable neurogenetic risk or shared environmental factors associated with schizophrenia. Schizophrenia is likely a polygenic disorder (Liu et al., 2017); therefore, it is difficult to speculate about the genetic or shared environmental influences that may underlie this temporal and occipital dysconnectivity. At a genetic level, it is possible that this reduced rs-FC was inherited through several common single nucleotide polymorphism (SNP) based variants, that act multiplicatively to result in abnormal rs-FC focused in occipital and temporal regions. For example, CDH13 genetic load from the cadherin family has been associated with rs-FC strength in healthy populations (Meda et al., 2014), and in neuropsychiatric disorders such as schizophrenia (Redies, Hertel, & Hubner, 2012). This gene mediates cell adhesion and intracellular signaling, playing a crucial role in the development of neural circuitry and synaptic function (Meda et al., 2014). It is possible that CDH13 and other implicated gene SNP variants are driving this reduced rs-FC in probands and UFM. As demonstrated in *Chapter 4*, this heritable phenotype alone does not lead to psychosis onset, or any observed functional impairment. Future research should investigate this sample genotypically to explore the relationship between common adverse environmental factors, genes or genetic variants and network connectivity.

Conversely, the connections that represented precipitating risk/illness effects predominantly involved temporal and frontal lobes. Dysconnectivity involving frontal and temporal regions is consistent with the known pathophysiology of schizophrenia, with a recent review finding the frontal lobe to be most involved in reduced connectivity irrespective of schizophrenia illness stage and independent of the neuroimaging method employed (Friston, Brown,

Siemerkus, & Stephan, 2016). Temporal lobe dysconnectivity has also been repeatedly associated with schizophrenia symptomatology, such as auditory hallucinations (Sommer, Clos, Meijering, Diederen, & Eickhoff, 2012) and thought disorder (Lui et al., 2009). Additionally, our results showed that the majority of these reduced functional connections were 'inter-lobe', suggesting that TRS is associated with long-range dysfunctional interactions between vital brain hubs. This disruption in functional integration may subsequently lead to further cognitive impairment (Sheffield & Barch, 2016) and the exacerbation of positive symptomatology (Rotarska-Jagiela et al., 2010) that is characteristic of schizophrenia and is often severe in TRS, such as persistent auditory hallucinations. It is possible that this reduced rs-FC may be a factor contributing to the development of the disorder, however, again, it is impossible to determine whether it preceded the onset of schizophrenia, or developed as a result of illness progression and medication effects.

5.2.3. Findings associated with resilience

To date, the majority of endophenotype research in schizophrenia has focused on identifying genetic, neurobiological and cognitive risk factors that may precipitate or perpetuate psychosis. More recently, there has been increasing recognition for the need to characterise putative factors that may confer resilience to developing the disorder. The concept of resilience in psychiatry is multidimensional, and traditionally, resilience was viewed as a psychological adaptation that was protective in the face of adversity or trauma (i.e. abuse) (Luthar, Cicchetti, & Becker, 2000). Current models of resilience are more dynamic, and emphasise the involvement of multiple systems (i.e. neuroanatomical, psychophysiological or neuropsychological) that may provide some form of protection against the development of a psychiatric disorder. We aimed to explore this concept of resilience in *Chapter 4*. Resilience was operationalized as any significant difference in network measures that was unique to the

UFM group relative to both the TRS and control groups, and thus may be a marker of resilience to the development of schizophrenia.

Approximately 5% of the functional connections that differed between groups were classified as resilience markers, and these connections predominantly involved temporal and subcortical regions. These connections were uniquely reduced in UFM, without significant differences between TRS and healthy controls. The present study, however, was cross-sectional, and therefore could not explicitly test for evidence of resilience against the development of schizophrenia. Further, the majority of these resilience connections were reduced in rs-FC in UFM relative to controls, which seems counter-intuitive when attempting to characterise measures of resilience against the development of a psychiatric illness, as one would expect them to be *increased*. Although our findings cannot be interpreted beyond a classification of connections that are significantly different between TRS, UFM and the general population, we proposed in *Chapter 4* three alternative explanations for the unique alterations in rs-FC seen in UFM. To briefly summarise these theories, we first hypothesised that reduced temporal and subcortical rs-FC may be an expression of susceptibility genes for schizophrenia, however given the reduction is much less severe and in different anatomical locations, the disorder may not emerge. Additionally, the fact that the frontal lobe was least implicated may indicate that UFM do not possess the necessary pathophysiology to precipitate onset of schizophrenia. Secondly, we proposed that the TRS group could have once displayed this same pattern of dysconnectivity, however antipsychotic treatment over time restored the reduced rs-FC to normal levels seen in controls, whereas UFM who have never been prescribed antipsychotics still show reduced rs-FC in these regions. This theory is supported by evidence in previous research of antipsychotic treatment normalising abnormal rs-FC in schizophrenia patients over time almost to levels observed in controls (F. Li et al.,

2016; Sarpal et al., 2015). Lastly, we hypothesised that if the genesis of the disorder is in temporal regions, additional reduced temporal rs-FC in UFM may be a protective mechanism to constrain or isolate pathology to the temporal lobes. Alternatively, these results can also be interpreted such that TRS patients showed some evidence of *increased* rs-FC relative to their UFM. Therefore, this could represent an inherited or environmental mechanisms at play that has contributed to overcoming a 'risk' scenario, and modify connectivity (i.e. increase rs-FC in TRS patients to levels observed in controls). For example, it has been shown that the positive impact of 'positive parenting' can ameliorate the negative effects of low socioeconomic status on brain development in adolescents (Whittle et al., 2017). Thus, investigating how/which environmental factors differed between UFM and TRS probands presents a way forward in exploring possible reasons for the observed increase in rs-FC in TRS individuals relative to UFM in specific brain regions. These theories however are highly speculative, and perhaps with a larger UFM sample size and a longitudinal design, more of these unique neural connections will emerge and further characterise network properties that could be conferring resilience against the emergence of psychosis in those at genetic risk of the disorder (Cropley & Pantelis, 2014).

5.2.4. Network topology in psychosis and in UFM

While we have described rs-FC in the previous section in relation to TRS and UFM, on the whole, and when considering our FEP results, rs-FC findings associated with schizophrenia are heterogeneous. This may suggest that rs-FC alone is not sensitive or specific enough to reliably identify and map neurobiological abnormalities that are characteristic to different illness stages of psychosis. To further interrogate resting-state networks at different illness stages of schizophrenia, we explored complementary measures of brain topology in conjunction with functional connectivity. Functional network topology reflects functional

brain organisation and efficiency of information transfer on a local and global level, therefore, characterising these properties in early and late schizophrenia in addition to UFM can provide further insight into the network pathophysiology that might be underlying psychosis.

Contrary to our hypothesis, we found no evidence that FEP networks differed topologically relative to controls in any of the graph theoretical measures tested. This finding is in line with results by Fornito et al. (2011), who investigated functional topology during a cognitive control task, and found intact functional network architecture in FEP individuals relative to controls (A. Fornito, Yoon, Zalesky, Bullmore, & Carter, 2011). Taken together, these results suggest that the functional efficiency of brain-wide information exchange, and local integration and communication of information at the nodal level (Fornito, Zalesky & Bullmore, 2016) is not impaired in some FEP samples relative to controls. Given that previous studies have shown abnormal structural topology in FEP (Crossley et al., 2017; Hu et al., 2016; Lord et al., 2012; Palaniyappan et al., 2016; Zhang et al., 2015), future research should study white matter connectivity in addition to structural network topology in this and other FEP samples to further determine whether structural network architecture is also intact. Our finding of no functional topological impairment in FEP may reflect their relatively good clinical state and prognosis and/or the efficacy of their treatment.

An alternative theory is that perhaps functional network topology is not a characteristic feature of the FEP illness stage. While some neurobiological factors may operate across several or all illness stages and transitions, other properties such as resting-state topology may be stage-specific, and more associated with the severe illness end of the psychosis continuum. Our results support this theory, given we found significant topological differences

in the TRS group relative to healthy controls. Further, our findings of abnormal network architecture in clinically healthy UFM, in contrast to our results of no differences in the FEP group suggest that abnormal topology and abnormal rs-FC are associated. Given that the network topology is estimated on a binary functional connectivity adjacency matrix, one would expect these two sets of measures to be intrinsically linked (Lynall et al., 2010). This theory is supported by previous work demonstrating correlations between rs-FC and graph metrics such as global and local efficiency in Schizophrenia (Lynall et al., 2010). Therefore, *Chapter 2* adds insight to the field of graph theory and functional connectivity research in schizophrenia by demonstrating that in this particular FEP cohort, topological properties and rs-FC both appeared to be intact, further supporting the relationship between the two fMRI measures.

In contrast to results in *Chapter 2*, in *Chapter 3*, we found the TRS group to show reduced global efficiency and increased local efficiency relative to healthy controls. Our finding of decreased global efficiency in the TRS group is in line with previous functional (Hadley et al., 2016; Lo et al., 2015; Lynall et al., 2010) and structural (Zalesky et al., 2011) findings in schizophrenia cohorts and indicates that topologically, TRS is associated with reduced global network integration that is not a result of the widespread reduction in rs-FC. The observed increased local efficiency in TRS patients has also been previously reported in schizophrenia groups (Hadley et al., 2016; Lynall et al., 2010), and suggests that a greater proportion of intra-modular connections may underlie the loss in long-range hub-to-hub connections. Increased local efficiency coupled with decreased global efficiency suggests that hub-to-hub connections are preferentially affected in TRS. Specifically, when hub-to-hub connections are eliminated, global efficiency is reduced in TRS relative to controls because these connections facilitate global integration between disparate modules. Moreover, when hub-to-

hub connections are eliminated, local efficiency is increased in TRS relative to controls (once normalised to degree-matched random networks) because the TRS networks comprise a greater proportion of peripheral, intra-modular connections, which support increased local efficiency. Interestingly, when comparing across the three groups in *Chapter 4*, we found UFM to show higher local and reduced global efficiency relative to TRS and controls. These findings beg the question: Why are these topological anomalies present in unaffected relatives, and why are they most pronounced in UFM?

Higher local efficiency means that for a given node and its connected neighbours, those neighbours are also more likely to be connected with each other. This results in a more clustered topology with a greater robustness to injury or disease (Lynall et al., 2010). It has been suggested that a highly-clustered topology means that more alternative paths exist between two given nodes in the event that one path (or connection) is destroyed as a result of brain trauma or disease, and this has the advantage of increasing the resilience of a given network to a random attack (Lynall et al., 2010). It is therefore possible that this increased robustness to random attack in UFM may be a compensatory mechanism to overcome the 'risk' associated with reduced rs-FC, and in turn represent a form of resilience against the development of schizophrenia. Relatedly, one theory on the etiology of schizophrenia suggests that individuals with the disorder show some neurobiological advantages that have enabled the disorder to be perpetuated throughout generations (Lynall et al., 2010). It is possible that the heritable neurogenetic product of increased local efficiency confers an evolutionary benefit that is most pronounced in UFM, and less pronounced in individuals with TRS relative to the healthy population.

Here, we debate the possibility that increased local efficiency might confer a protective

mechanism, however, one might question why we also see the greatest decrease in global efficiency in UFM, which generally implies poorer global integration and communication within a network. As discussed in *Chapter 4*, small-worldness is a balance between integration (global efficiency) and segregation (local efficiency). It could be that tipping the balance towards one side, in this case, towards local efficiency, may compromise global efficiency. Perhaps the most important protective factor is an increase in local efficiency, despite a consequential decrease in global efficiency, that is, the benefit of increased resilience (i.e. increased local efficiency) outweighs the disadvantage of reduced global integration. The question then becomes, does this decrease in global efficiency have functional implications for this UFM group? The UFM group did not significantly differ from healthy controls in IQ, however it is indeed possible that they show deficits in specific cognitive domains that require higher level executive functioning. Global efficiency has been associated with cognitive ability in healthy individuals and in individuals with schizophrenia (Sheffield et al., 2017; Sheffield et al., 2015), thus, further investigation into the effects of disturbed long-range connections found in this UFM group would be of great interest.

5.3. Strengths and contribution to current knowledge

This thesis provides a number of important research outcomes that contribute to the current knowledge surrounding the neurobiology and etiology of psychosis at different illness stages. Throughout the thesis, we chose to investigate rs-FC using a whole-brain data driven approach, in order to maximise inference and minimise bias. This method has been shown to be a powerful tool to map disturbances in rs-FC across the whole brain in healthy and other psychiatric populations, as it enables an explorative, holistic representation of functional connectivity across the entire brain. We investigated whole-brain networks further by exploring resting-state network-architecture using well established graph theory measures to

probe deeper into the potential underlying neuropathophysiology associated with schizophrenia. The empirical chapters of this thesis used reliable and consistent methodological approaches to analyse the available data, and this enabled us to robustly examine the rs-FC and topological abnormalities that are evident in early and late schizophrenia, and in UFM of individuals with schizophrenia.

Firstly, our study is the first to report no significant differences in rs-FC in FEP individuals relative to healthy controls. Although our results do not support the prevailing findings of abnormal rs-FC in FEP, they are a valuable contribution to the scientific field, and add encouraging insight to the existing literature. It is important for the field of early intervention and early psychosis research to know that not all FEP samples show neurobiological anomalies relative to healthy controls. From this, exploration into the factors that contributed to this FEP group showing no observed abnormalities can be studied. Further, our study was the first to probe deeper into resting-state functional networks in FEP and investigate network topology in conjunction with functional connectivity, and whether these properties change abnormally over time. Given that the early illness stage of psychosis is dynamic and heterogeneous, our findings of no abnormal change in rs-FC or network topology over 12-months in this cohort is an important result and addition to early psychosis research. Rather than viewing FEP populations as having inevitably poor prognoses with evident neurobiological abnormalities and deterioration, the current findings support the notion that the course and severity of early psychosis may be more fluid, malleable, and in the current sample, more optimistic.

Secondly, despite the clinical relevance of TRS, very few neuroimaging studies have focused on this chronic late illness stage of schizophrenia. Furthermore, our study was the first to

investigate network architecture in a TRS cohort and demonstrate that in addition to widespread reduced rs-FC, TRS patients also show disturbed network topology relative to healthy controls, specifically, reduced global efficiency and increased local efficiency.

Thirdly, the findings from this thesis further support the theory that risk-related disrupted rs-FC and network topology may be, in part, genetically determined and heritable. The results of *Chapter 4* enabled us to distinguish neurobiological precipitating risk and/or illness effects of schizophrenia, from heritable/shared environmental risk markers that alone, do not appear to cause any observable psychiatric or functional deficits in UFM. These findings further support the notion that an interplay between biological and environmental factors contribute to the onset and establishment of the disorder (Gogtay, Vyas, Testa, Wood, & Pantelis, 2011; Pantelis et al., 2005).

5.4. Limitations and future directions

The studies included in this thesis had various limitations that restricted the number of inferences that can be made about the integrity of rs-FC and topology at different illness stages of psychosis. A major caveat was the small sample size of both the FEP and UFM cohorts. Unfortunately, due to the high attrition rate, we were only able to analyse 12-month follow-up data for a proportion of the FEP participants (n=14 out of n=29). With this said, however, our cohort size at baseline was comparable and even larger than that of other FEP studies that reported abnormal rs-FC (Alonso-Solis et al., 2012; Anticevic et al., 2014; Ebisch et al., 2014; A. Fornito et al., 2013; Guo et al., 2017; Li, Xu, Zhang, Hoptman, & Zuo, 2015; T. Li et al., 2016; Yoon et al., 2015; Zhou, Tan, Tang, & Chen, 2010), indicating that our finding of no differences (at least at baseline) was not due to low power. Relatedly, our UFM sample size was also quite small (n=16). There are three main problems associated with low

power that may contribute to producing unreliable findings: low chance of finding true effects; low positive predictive value when an effect is claimed and an exaggerated estimate of the effect when a true effect is found (Button et al., 2013). In regards to our FEP study, it is possible that the small sample size resulted in an inability to find any true effects of abnormal change in network measures over 12-months. Moreover, the stringent brain-wide corrections for multiple comparisons provided excellent control for false positives, however it was consequently more difficult to avoid false negatives (Radua et al., 2012). Similarly, it is also possible that our relatively small UFM sample may have resulted in low positive predictive value or an exaggerated estimate of the results found. However, a study of a phenotypically homogenous patient cohort is an important advantage that maximises the likelihood of reproducibility despite small sample size, as demonstrated with replication of our findings with an alternative parcellation atlas. Future research should endeavor to investigate these cohorts in larger sample sizes, to determine with better accuracy what true neurobiological deficits are developing during the early illness phase, and the heritability of abnormal rs-FC and topology in UFM.

A second limitation of this thesis was our inability to investigate network measures in a chronic, treatment-responsive schizophrenia group. In terms of the clinical staging model, it would have been of great benefit to explore functional connectivity and topology in a schizophrenia cohort that sits somewhere between FEP and TRS on the psychosis continuum. This would have enhanced our understanding of the neurobiological underpinnings and effects of schizophrenia from one illness stage to the next, with less of a gap in illness duration and chronicity between groups. However, this data was not available. Comparing TRS with healthy controls limits the inferences that can be made, as differences found between groups cannot necessarily be translated as markers of ‘treatment resistance’ but

instead may reflect neurobiological abnormalities found in schizophrenia in general. Furthermore, given all TRS participants were on clozapine at the time of assessment, the effects that this atypical antipsychotic had on rs-FC and network efficiency is difficult to characterise. Therefore, to further understand the neurobiology of TRS distinct from the effects of clozapine, future research should endeavour to investigate rs-FC and topology in a TRS group relative to a treatment-responsive schizophrenia cohort that are not on clozapine.

A third limitation of this thesis was the significant difference in age between the UFM and TRS groups. This was because the majority of UFM comprised parents of probands, making it impossible to match the groups in terms of age. Although we controlled for this confound statistically in every analysis, we cannot rule out the potential effects that age has on rs-FC and topology. Given siblings are closer matched in age than parents are with children, future research should investigate rs-FC and topological risk and resilience markers in siblings of probands, to eliminate any potentially confounding effects of age.

A final limitation worth discussing was the lack of observed relationships between network measures and symptomatology in all three empirical chapters. As previously discussed in *Chapter 2*, the FEP cohort had lower total symptom scores relative to previous FEP studies, placing them in a mild to moderately ill bracket. This could be indicative of effective response to antipsychotic medication and/or psychosocial therapy, and findings of no network abnormalities and no relationship with symptomatology might reflect this relatively 'positive' clinical picture. Conversely, the lack of an observed association between symptomatology and network measures in the TRS group reported in *Chapter 3* and *Chapter 4* may be due to different causes. Given the chronicity of the TRS sample, and the widespread reduction in rs-FC observed, the lack of association may represent a downstream

ceiling effect of brain connectivity on symptom severity due to factors such as prolonged illness duration and long-term medication effects. Future studies should endeavor to explore whether and how reduced rs-FC and network efficiency is associated with clinical heterogeneity of symptoms in TRS, and whether this differs in comparison to treatment-responsive schizophrenia.

5.5. General conclusion

Through mapping rs-FC and functional topology across different stages of schizophrenia, this thesis provided a number of novel findings that contribute original insight about the pathophysiology, etiology and heritability of psychosis and schizophrenia. The work demonstrated that the latest most severe stage of psychosis, TRS, is associated with widespread reduced rs-FC, and that milder, yet similar patterns of dysconnectivity were observed in UFM, implying a genetic/shared environmental root to some, but not all of the observed network abnormalities. Network topology differed relative to healthy controls in both UFM and schizophrenia patients, suggesting that in addition to reduced functional connectivity, functional network architecture is also disturbed in late psychosis, and again, results suggest a genetic/shared environmental basis for this characteristic. Our finding of no significant difference in rs-FC or network topology in our FEP sample supports the notion that there is a differentiation between biological processes occurring in early and late psychosis, and suggests that a subgroup of individuals' rs-FC is unaffected in the FEP stage, providing further support for the clinical staging model of psychotic illness. Thus, the work included in this thesis provides fertile ground for future studies to further characterise and elucidate the neurobiology underlying psychosis.

5.6. References

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