Brain structural and white matter changes in first-episode schizophrenia and their demographic, clinical and cognitive correlates

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe on any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes 3 original papers published in peer reviewed journals (2 as first author and one as second author) and 2 unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the cases where this is not the case a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

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ABSTRACT

In schizophrenia, decreased brain volume and altered cortical thinning (especially in the frontal and temporal areas), as well as white matter deficits are described at the first-episode. The relationship between these brain measures and clinical symptoms, whether there is progression, and the extent to which antipsychotic medication contribute to or mitigate those changes remains unclear. The aim of this PhD was to examine cortical thickness, brain volume (cortical, subcortical, white matter) and diffusion tensor imaging data, looking at the relationship between these brain measures and clinical variables in the first year of schizophrenia treatment.

This PhD focused on the MRI subcomponent of a larger prospective longitudinal study in first-episode schizophrenia (FES) patients treated with flupenthixol decanoate medication. The thesis integrates the findings of five journal manuscripts that each focused on a clinically relevant neuroimaging question that emerged as we assessed patients in the parent study, namely insight, childhood trauma, neuroimaging predictors of symptom expression, and antipsychotic related brain changes.

In our first manuscript, baseline fractional anisotropy (FA) in a number of white matter tracts predicted poorer total insight in 89 FES patients, with a predilection for tracts associated with cortical midline structures. In our second manuscript, the 'symptom misattribution' domain of clinical insight was associated with significantly thinner left anterior cingulate and left rostral middle frontal cortices. Our studies address a need for research in larger samples in FES to better understand the neurobiology of insight in schizophrenia. In our third manuscript, baseline FA deficits in cortico-limbic circuitry was associated with childhood trauma in 53 FES patients compared to 51 controls, and there were differential effects of childhood emotional neglect (increased FA) and sexual abuse (decreased FA) on white matter in patients. To our knowledge, at the time of manuscript submission for publication, this was the first study examining the relationship between childhood trauma, FA and FES.

For our fourth manuscript, baseline brain measures in 54 FES patients were differentially associated with state and trait symptom expression over 12 months, with global gray matter significantly associated with sensory integration and verbal learning trait scores, cortical volume with verbal learning trait scores, cortical thickness with social and occupational functioning trait scores, and white matter volume with motor coordination state scores. Of potential relevance to patient care is that these neuroimaging deficits at initial presentation in FES may predict enduring trait deficits in cognition, functioning and neurological soft signs. For our final manuscript, total antipsychotic dose was a predictor of substantial cortical brain volume reductions over twelve months of treatment in 23 antipsychotic naïve patients

compared to 53 matched controls. Our finding of a significant relationship between antipsychotic dose and cortical volume reduction in this study strongly suggests causality.

Future research directions stemming from this PhD include further exploration of our longitudinal data, strengthening our clinical assessments of insight and childhood trauma, connectomic analyses, a multi-modality neuroimaging approach, hippocampal subfield segmentation, and broadening our international collaborations.

OPSOMMING

In skisofrenie word verminderde breinvolume en veranderde kortikale verdunning (veral in die frontale en temporale areas) sowel as witstof-tekorte reeds by eerste aanvang beskryf. Die verwantskap tussen hierdie brein afmetings en kliniese simptome, of daar voorsetting is en die mate waartoe antipsigotiese medikasie hierdie veranderinge vererger of versag is onduidelik. Die doelwit van hierdie PhD was om kortikale dikte, breinvolume (kortikaal subkortikaal, witstof) en “diffuse tensor” beeldingsdata te ondersoek deur te kyk na die verhouding tussen hierdie brein afmetings en kliniese veranderlikes gedurende die eerste jaar van skisofrenie behandeling.

Hierdie PhD se fokus was die MRB sub-afdeling van ‘n omvattende prospektiewe longitudinale studie in eerste aanvangs skisofrenie (EAS) pasiënte behandel met flupenthixol dekanoaat medikasie. Die tesis integreer die bevindinge van vyf joernaal manuskripte wat elkeen gefokus het op ‘n klinies relevante neurobeeldingsvraagstuk soos na vore gekom tydens die assessering van pasiënte in die oorhoofse studie; naamlik insig, trauma tydens kinderjare, neurobeeldingsvoorspellers van simptoom uitdrukking, en antipsigotika-verwante breinveranderinge.

In ons eerste manuskrip het basislyn fraksionele anisotropie (FA) in ‘n aantal witstoftrakte laer globale insig in 89 EAS pasiënte voorspel met ‘n voorkeur vir trakte geassosieer met midlyn kortikale strukture. In ons tweede manuskrip was die “symptom misattribution” domein van kliniese insig geassosieer met ‘n beduidend dunner linker singulaat en linker rostrale middel frontale kortises. Ons studies spreek tot die behoefte vir groter studiegroepe in EAP om sodoende die neurobiologie van insig in skisofrenie beter te verstaan.

In ons derde manuskrip was basislyn FA tekorte in kortiko-limbiese bane geassosieer met trauma in die kinderjare in 53 EAS pasiënte in vergelyking met 51 kontroles. Daar was ook differensiele effekte op witstof vir verwaarlosing in die kinderjare (verhoogde FA) en seksuele misbruik (verminderde FA) in pasiënte. Toe die manuskrip vir publikasie voorgelê is, was dit, na die beste van ons wete, die eerste studie wat die verwantskap tussen trauma in die kinderjare, FA en EAP ondersoek het.

Vir ons vierde manuskrip was basislyn brein afmetings in 54 pasiënte differensieël geassosieer met toestand en eienskap simptoom uitdrukking oor 12 maande, met globale grysstof beduidend geassosieer met sensoriese integrasie en verbale leer eienskap tellings, kortikale volume en verbale leer eienskap tellings; kortikale dikte met sosiale- en werksfunksionering eienskap tellings en witstof volume met motor koordinasie toestand tellings.. Dat hierdie neurobeeldingsuitvalle wat teenwoordig is met die eerste episode dalk

blywende eienskap uitvalle in kognisie, funksionering en neurologiese sagte tekens mag voorspel is potensieel van waarde vir pasiëntsorg. Vir ons laaste manuskrip het die totale antipsigotika dosis aansienlike kortikale breinvolume vermindering voorspel oor 12 maande van behandeling van 23 antipsigotika naïewe pasiënte in vergelyking met 53 ooreenstemmende kontroles.Our finding of a significant relationship between antipsychotic dose and cortical volume reduction in this study strongly suggests causality. Ons bevinding in hierdie studie van ‘n beduidende verhouding tussen antipsigotika dosis en kortikale volume vermindering suggereer sterk oorsaaklikheid.

Toekomstige navorsingsgeleenthede wat voortspruit uit hierdie PhD sluit in: verdere ondersoek van ons longitudinale data, geleenthede om kliniese assessering van insig en trauma in kinderjare te versterk; “connectomic” analises, multi-modale neurobeeldingsbenaderinge, hippokampale subveld segmentasie en die uitbreiding van ons internasionale samewerkings-ooreenkomste.

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This work is dedicated to those who live with schizophrenia, who rise every day with dignity and hope despite adversity.

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CHAPTER 1: INTRODUCTION

CONTEXTUALISING GLOBALLY AND LOCALLY RELEVANT FIRST-EPISODE SCHIZOPHRENIA NEUROIMAGING RESEARCH

This chapter begins with an overview of select topics which serves to contextualise the thesis rather than provide a comprehensive review of magnetic resonance imaging findings in first-episode schizophrenia. This overview is followed by the aims and objectives of the thesis after which I describe the parent study in which my PhD is nested. To conclude, I provide a brief summary of the subsequent chapters and describe how the included journal manuscripts serve as a coherent body of work.

SCHIZOPHRENIA: BURDEN OF DISEASE

Schizophrenia is a severe, disabling illness characterised by psychosis, apathy and withdrawal, and cognitive impairment, which result in deterioration in social and occupational functioning (van Os and Kapur, 2009). Schizophrenia affects approximately 0.3% to 0.7% of the population at some point in their life and contributes significantly to the global health burden because of its early onset, devastating effects and chronic course (WHO, 2011). The aetiology of schizophrenia remains poorly understood and neuroimaging techniques, such as magnetic resonance imaging (MRI), have enabled investigation of possible pathological processes involved in the illness.

THE CONTRIBUTION OF NEUROIMAGING TO UNDERSTANDING SCHIZOPHRENIA

Over 100 years ago Kraepelin theorized that damage to the cerebral cortex, in particular the prefrontal and temporal cortex, plays a fundamental role in the pathogenesis and progression of schizophrenia (Shenton et al. 2010). Interest in brain abnormalities was rekindled in 1976 when the first computed tomography (CT) study described dilated lateral ventricles in schizophrenia (Johnstone et al. 1976). A considerable number of CT and MRI studies came after. The first MRI study in schizophrenia was published in 1986 (Andreasen et al. 1986) and although resolution in early MRI studies was poor relative to MRI scans of today, it still greatly surpassed that of CT scanning with regards to the ability to differentiate between gray and white tissue and to assess subtle changes in brain volume. In recent years, the cost of MRI has decreased, spatial resolution has increased, magnet strength has progressed and the entire brain can be scanned in a relatively short period of time. MRI is currently the neuroimaging modality of choice in schizophrenia.

Neuroimaging, particularly MRI, has extensively expanded our knowledge of schizophrenia although we are left with many important questions. What is the degree of pathology evident at illness onset? Are there evolving brain deficits over the early illness course? Do brain deficits predict therapeutic outcome? Addressing these questions could contribute to a better understanding of the core pathophysiology of schizophrenia and more effective intervention for the illness (Gong et al. 2015).

MAGNETIC RESONANCE IMAGING

What is MRI?

MRI is a medical scanning technique that, at an elemental level, uses a strong magnet and a radio transmitter and receiver to generate images. Hydrogen protons in the body behave somewhat like bar magnets spinning along their north-south poles with their axes randomly aligned. When a person is placed in an MRI scanner, strong magnetic field leads to the protons' axes all lining up which creates a magnetic vector along the axis of the MRI scanner. When pulses of radio waves are added to the magnetic field, the magnetic vector is deflected. When the pulse is switched off, the magnetic vector relaxes back to a resting state and a radio wave signal is emitted. These radio signals are plotted to create an MR images. To distinguish protons from one another, MRI's manipulate both the magnetic field and the pulse sequence of radio wave energy so that different tissue types (e.g. gray matter, white matter) emit slightly different radio signals in order to build a three-dimensional image (Berger 2002).

Freesurfer and automated processing of MRI

The use of automated post-processing computer software is a relatively recent way to classify and quantify brain tissue in neuroimaging studies. These software packages automatically remove non-brain tissue, align the brain to a common system and segment the brain into different tissue types based on MRI signal intensity (Ashburner and Friston 2000). An advantage of computerised image analyses is that a large number of images can be processed in a shorter period of time. This is especially important since MRI costs and image acquisition times are decreasing and image datasets are growing. By reducing the risk of measurement bias inherent to humans tracing brain structures, computerised post-processing also increases consistency and sensitivity of measurement (Morey et al. 2010). Freesurfer (http://surfer.nmr.mgh.harvard.edu/) is a widely used open source automated processing software package that was used for the measurement of brain volume and cortical thickness in this PhD study.

Cortical thickness

Cortical thickness is an indication of the size, density, and arrangement of neurons, neuroglia and nerve fibres (Narr et al. 2005), and abnormalities in cortical thickness may suggest fundamental neuropathological abnormalities in the intrinsic structure and integrity of cortical laminae (Kuperberg et al. 2003). Cortical thickness may be a more sensitive measure of

subtle gray matter abnormalities in schizophrenia than volumetric studies, particularly in the early phase of the disorder (Xiao et al. 2013). Cortical thickness studies in schizophrenia, particularly those examining for a relationship with clinical factors are few and results are inconsistent. This may in part be due to studies with relatively small sample sizes assessing multiple brain regions each with small effect sizes in the presence of large inter-individual differences. Also, potential confounding effects of age, and antipsychotic exposure in patients with chronic schizophrenia need to be taken into account.

Diffusion Tensor Imaging (DTI)

Brain connection irregularities within and between brain regions may constitute core pathology in schizophrenia (Wheeler and Voineskos 2014) and white matter forms the basis of this connectivity. The microstructural properties of white matter tracts are usually studied in vivo with DTI, an approach that provides a number of measures of white matter integrity, of which Fractional Anisotropy (FA) is probably the most commonly reported (Wheeler and Voineskos 2014). FA values are thought to reflect both myelination and organization of fibre tracts that form the basis of brain connections (DeRosse et al. 2014). A meta-analysis of DTI findings in first-episode schizophrenia reported reduced FA predominantly in the right deep frontal and left temporal lobes in patients compared to controls although results of individual studies are quite variable. This may be because FES changes are subtler than in chronic schizophrenia and may only manifest later in the disorder, possibly because of medication effects (Fitzsimmons et al. 2013), or because sample sizes in FES are frequently small and studies are heterogeneous from a methodological perspective.

Baseline MRI findings in first-episode schizophrenia

Brain structural abnormalities in schizophrenia are described at both the first-episode and in the chronic phase of the illness (Chan et al. 2011) and considerable evidence suggests that schizophrenia is a brain disease characterised by reductions in gray matter and white matter (Bora et al. 2011). A meta-analysis of brain volume in 8327 patients with schizophrenia including 771 medication naïve, recent onset patients found a small but significant decrease in intracranial volume in patients compare to controls and nearly twice as great a reduction in total brain volume. Total brain volume decrease was attributable to a decrease in gray matter (white matter volume decrease was similar to that of intracranial volume). Effect sizes of total brain volume and gray matter volume in medicated patients were 30% lower than in antipsychotic naïve patients and that of white matter volume was comparable. The authors argue that white matter volume reduction is present before antipsychotic treatment is started and progresses little while gray matter volume reduction worsens with illness duration and higher dose of antipsychotic treatment (Haijma et al. 2012).

Studies investigating cortical thickness are relatively few and findings are inconsistent. Findings of reduced thickness in bilateral dorsolateral prefrontal cortex thickness in first episode and chronic schizophrenia has been described (Wheeler et al. 2013). Negative results in a first-episode schizophrenia study have also been reported (Goghari et al. 2013), as have widespread differences in cortical thickness (including both thinning and thickening) in a large sample of antipsychotic naïve patients (n=128) compared to controls (n=128) (Xiao et al. 2013). Padmanabhan and colleagues found that positive symptoms in particular were correlated with gray matter volume and cortical thickness in frontal and temporal regions although the magnitude of correlations were low (Padmanabhan et al. 2014).

Studies of white matter in first-episode schizophrenia indicate widespread abnormalities including the corpus callosum (Rotarska-Jagiela et al. 2008), fornix (Kuroki et al. 2006), cingulum (Kubicki et al. 2003), uncinate (Mandl et al. 2013) and inferior longitudinal fasciculus (Ashtari et al. 2007). Although there are inconsistencies in results (Melonakos et al. 2011), there is evidence for a predilection for frontal and temporal deep white matter (Ellison-Wright and Bullmore 2009). White matter changes may be linked to the development of schizophrenia and appear to be in keeping with the neurodevelopmental model of the illness (Sommer and Kahn 2014;Collin et al. 2013). On the other hand, volume changes and thinness of the cortex (particularly frontal and temporal) appear to be related to the illness itself and possibly associated with outcome and antipsychotic medication (Sommer and Kahn 2014) which is more in keeping with the neuroprogressive (or neurodegenerative) model of the illness. Also, somewhat counter-intuitively, there is some evidence for increased regional gray matter volume (e.g anterior cingulate, orbitofrontal gyrus, thalamus (Ellison-Wright et al. 2008;Szeszko et al. 1999)) as well as increased cortical thickness in the temporal lobe bilaterally prior to treatment onset (Xiao et al. 2013). This increase may be due to a deficit in normal pruning, or acute neuroinflammation, preapoptotic osmotic changes or hypertrophy (Gong et al. 2015;Xiao et al. 2013).

Although studies have described correlations between regional brain volume deficits and positive and negative symptoms, the relationships between clinical symptoms and brain pathology has been, at best, fairly modest (Gong et al. 2015). The limited success has in part been because a number of conceptual and methodological challenges undermine the efforts to link symptom severity with neurobiological correlates. These challenges include the confounding effects of medication, small sample size, the use of different instruments to measure symptoms and the failure to distinguish state-trait effects (Mathalon and Ford 2012).

Neuroimaging predictors of outcome in first-episode schizophrenia

The baseline MRI findings in schizophrenia described above offers some hope that these deficits may serve as potential biomarkers for treatment outcome. A review of eleven studies by Dazzan and colleagues found that reductions in medial temporal and prefrontal volumes and in the networks that connect them may be related to poor symptomatic and functional outcomes (Dazzan et al. 2015). The authors also noted that there are relatively few studies related to the topic and results are inconsistent with a large variability in findings.

Longitudinal neuroimaging findings in first-episode schizophrenia

There is growing evidence from longitudinal studies that global gray matter volume reduction in schizophrenia is progressive and is most pronounced in the early phase of the illness (Liberg et al. 2016;Pantelis et al. 2005). Progressive neocortical gray matter volume loss and ventricular enlargement seems to be particularly pronounced with a relative sparing of white matter volume (Nakamura et al. 2007). It has been suggested that while schizophrenia may arise from a neurodevelopmental diathesis, its pathophysiology may be progressive after the onset of the illness (Lieberman et al. 2005). Decreased brain volume (Hulshoff Pol and Kahn 2008) and altered cortical thinning (van Haren et al. 2008) especially in the frontal and temporal areas progress as the illness worsens. It remains unclear the extent to which clinical outcome and antipsychotic medication contributes to or mitigates these brain changes (Vita et al. 2015).

Although schizophrenia patients with high levels of negative symptoms (Hulshoff Pol and Kahn 2008) and disorganization (Collin et al. 2012) exhibit more pronounced decreases of brain volume longitudinally, studies examining the relationship between symptomatology and progressive brain volume changes are limited and have yielded inconsistent findings. In general, most studies examining clinical parameters in relation to possible brain volume changes over time find no significant association (Sommer and Kahn 2014). However, there is some evidence that longitudinal brain changes are related to outcome and, at least in part, to antipsychotic medication (Vita et al. 2015).

AIMS

The overall aim was to examine brain structure and white matter in a cohort of patients with first-episode schizophrenia, and to assess the relationship between these brain measures and multiple demographic, clinical, and cognitive variables.

OBJECTIVES

The thesis focused on the following MRI brain measures:

brain volume (cortical, subcortical, white matter), cortical thickness, fractional anisotropy.

The objectives of the study addressed the overall aim by examining:

1. The difference in baseline brain measures between first-episode schizophrenia patients and matched community controls.
2. The relationship between brain measures and demographic, clinical and cognitive variables in patients experiencing a first-episode of illness.
3. Whether baseline brain measures are predictive of clinical and cognitive outcome for schizophrenia at 12 months.
4. Changes in brain measures over time after a first-episode of schizophrenia.

OVERALL DESCRIPTION OF THE PROJECT

Overview of parent study

This PhD was a sub-study of a larger prospective longitudinal study investigating clinical, biological and functional aspects of outcome in first-episode schizophrenia.

For inclusion in the parent study, patient participants had to be aged 16-45 years and experiencing a first-episode of schizophrenia, schizophreniform disorder, or schizoaffective disorder. First-episode patients were recruited from in- and out-patient services at Tygerberg Hospital and related community clinics. The control group was recruited from the same community as the patient group and matched for age, and sex. Patients and controls were excluded if they had a serious or unstable general medical condition, mental retardation, overt substance abuse and less than 7 completed years of schooling.

Patients were excluded if they had lifetime exposure to > 4 weeks of antipsychotic medication or were previously treated with a long-acting depot antipsychotic. The parent study was registered on the South African National Clinical Trials Register (www.sanctr.gov.za/SAClinicalTrials/tabid/169/Default.aspx), trial number DOH-27-0710-1957. The study was conducted according to the principles of the Declaration of Helsinki 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/). After the study procedures were fully explained in accordance with the ethical guidelines of the Stellenbosch University Human Research Ethics Committee, participants provided written informed consent which encompassed all aspects of my PhD study.

Outline of the thesis

My study focused specifically on the MRI component of the parent study. I examined cortical thickness, brain volume and white matter (diffusion tensor imaging) MRI data, looking at the relationship between these measures and clinical variables in the first year of treatment for schizophrenia. This thesis integrates the findings of five journal articles, three of which have been published (two with the PhD candidate as first author and one as second author), and two publications that are under review at international peer reviewed journals (both with the PhD candidate as first author).

Of the 126 patients recruited into the parent study, the first 25 were excluded from the neuroimaging component because of an approval delay for scans. The number of participants in each journal article differs according to the research question and the available data for that question. For example, Chapter 6 included patients who were antipsychotic naïve only, Chapter 5 did not include controls, childhood trauma data was not available for all participants (Chapter 4). Each journal article details the inclusion and exclusion criteria relevant to that research question.

The coherence of this thesis is based on three themes that run through the included journal manuscripts. Firstly, under the guidance of my supervisors, for each first author manuscript I led the process of deciding on a research question, assisted with pre- and post-processing of scans, performed the statistical analysis, did clinical assessments of participants and controls, drafted, edited, finalized and submitted journal manuscripts for publication.

Secondly, there was an opportunity with each journal article for myself and the schizophrenia neuroimaging team to develop skills in neuroimaging and statistical analysis. This includes skills training in DTI analysis for myself and other schizophrenia researchers (Chapter 2 and 4 are the first DTI based articles published from our unit). We also learnt statistical techniques novel to our team, such as least angle regression (chapter 2), hierarchical regression (chapter 5) and whole brain analysis (chapter 4).

And thirdly, each article fulfils at least two of my study objectives while being fundamentally clinically relevant at a local and global level. My objectives for the PhD study were broad to provide the scope to address neuroimaging research questions that were relevant to the clinical needs of patients as they emerged in the parent study. Some of the clinical concerns that emerged as we assessed participants clinically included the high prevalence of childhood trauma in our sample (chapter 4), the challenge of treating a patient with impaired insight (chapter 2 and 3), the effects of uninterrupted medication administration that was

given in our parent study (chapter 6), and the difficulty predicting which patients will have enduring clinical symptoms (chapter 5). In this respect, each manuscript is linked by these central themes.

Chapter 2 is a published journal article that examined for white matter fractional anisotropy differences between 89 first-episode schizophrenia patients and 98 community controls, and identified those differences associated with impaired insight in patients. At the time of publication, no other known study had examined the relationship between insight and white matter in first-episode schizophrenia, and findings from chronic schizophrenia were inconsistent. We used a machine learning algorithm to examine white matter regions across the entire brain as delineated by tract based spatial statistics (TBSS) for predictors of insight.

Chapter 3 is a published journal article and extends the findings of Chapter 2. Symptom attribution involves the awareness of symptoms as well as the ability to relabel symptoms as pathological, which is thought to require a higher level of insight function (Andreasen et al. 1986). In contrast to the widespread network of brain areas implicated in other insight domains (as described in Chapter 2), symptom attribution may more specifically relate to the frontal lobe. This paper uses a region of interest approach to examine the relationship between cortical thickness in 12 prefrontal regions and symptom attribution.

Chapter 4 is an article undergoing peer review at an international journal. This is the first known study to examine the relationship between childhood trauma and white matter in first episode schizophrenia. The article also reflects the evolution of clinical focus to childhood trauma within our larger schizophrenia research team.

Chapter 5 is an article also undergoing peer review at an international journal. Here, we investigated for baseline brain volume predictors of trait (endpoint) and state (change) related clinical symptoms. Enduring clinical symptoms in patients with schizophrenia constitute a large proportion of the costs and burdens of the illness. There are currently no useful biomarkers for predicting which patients will have ongoing clinical symptoms and results from other studies are inconclusive.

Chapter 6 is a published journal article which I second authored. Brain volume reduction is well documented in schizophrenia (Haijma et al. 2012) and longer-term studies provide evidence that these reductions are progressive (Vita et al. 2015). In this article, we examined for changes in cerebral gray and white matter volume over the first year of treatment in a subset of antipsychotic naïve patients. We compared brain volume in these patients to that of

matched community controls and investigated whether brain changes in patients were associated with treatment and outcome.

The concluding chapter (7) seeks to integrate the results of each journal article and provides a narrative synthesis of the results and summary of contributions in the context of the study objectives. This chapter also presents a set of concluding recommendations and suggestions for future research.

I have included the published version of the journal articles if available (Chapters 2, 3 and 6). Chapters 4 and 5 are undergoing peer review and are formatted in keeping with the requirements of the respective journals. However, for uniformity I have formatted the references of all unpublished chapters in the same style (Chapter 1, 4, 5, 7).

Manuscripts	Publication status	Number of participants	Imaging modality	Objectives addressed
Chapter 2: Insight and white matter fractional anisotropy in first-episode schizophrenia	Published First Author	89 Patients 98 Controls	DTI	1,2
Chapter 3: Symptom attribution and frontal cortical thickness in first-episode schizophrenia	Published First Author	92 Patients 93 Controls	Cortical thickness	1,2
Chapter 4: Childhood trauma associated white matter abnormalities in first-episode schizophrenia	Under review First Author	53 Patients 51 Controls	DTI	1,2
Chapter 5: Associations between global brain measures and state- and trait-related symptom expression of schizophrenia	Under review First Author	54 Patients	Brain volume, cortical thickness	2,3

Chapter 6: Brain volume changes over the first year of treatment in schizophrenia: relationships to antipsychotic treatment	Published, Second Author	23 Patients 54 Controls	Brain volume	3,4

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CHAPTER 2:
INSIGHT AND WHITE MATTER FRACTIONAL ANISOTROPY IN FIRST-EPISODE SCHIZOPHRENIA

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Insight and white matter fractional anisotropy in first-episode schizophrenia

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ABSTRACT

Impaired insight is a hallmark feature of schizophrenia. Structural studies implicate predominantly prefrontal, cingulate, cuneus/precuneus, and inferior temporal brain regions. The cortical midline structures (CMS) are also implicated in functional studies primarily through self-reflective processing tasks. However, few studies have explored the relationship between white matter tracts and insight in schizophrenia, and none in first-episode schizophrenia (FES). Here, we examined for fractional anisotropy (FA) differences in 89 minimally treated FES patients and 98 matched controls, and identified those FA differences associated with impaired clinical insight in patients. We found widespread FA reduction in FES patients compared to controls. Poorer insight in patients was predicted by lower FA values in a number of white matter tracts with a predilection for tracts associated with cortical midline structures (fronto-occipital, cingulate, cingulate hippocampus, uncinate, anterior corona radiata), and more severe depressive symptoms. The association between FA abnormalities and insight was most robust for the awareness of symptoms and illness awareness domains. Our study implicates a network of tracts involved in impaired insight in schizophrenia with a predilection for the CMS. This study is a first step in delineating the white matter tracts involved in insight impairment in schizophrenia prior to chronicity.

1. Introduction

Impaired insight is a hallmark feature of schizophrenia and is associated with poor treatment adherence (Yalcin-Siedentopf et al., 2014; Rüsch et al., 2009), a more severe course of illness (Johnson et al., 2012; Hoy et al., 2011) and poorer outcome in multiple symptom and functional domains (Margariti et al., 2015; Mohamed et al., 2009). The clinical model of insight encompasses illness awareness, treatment adherence, and the ability to attribute symptoms as pathological as defining features (David, 1990). As such, insight has become a focus of interest as a predictor of outcome, as well a therapeutic target for both pharmacological and psychological interventions (Lysaker et al., 2013).

Structural neuroimaging studies describe a significant association between impaired insight and a range of brain abnormalities involving predominantly prefrontal (Sapara et al., 2007; Shad et al., 2004; Shad et al., 2006), anterior and posterior cingulate (Ha et al., 2004), cuneus and precuneus, and inferior temporal brain regions (Ha et al., 2004). Illness awareness may be mediated by alterations in the dorsolateral prefrontal cortex (DLPFC) and middle frontal gyrus (Shad et al., 2004; Shad et al., 2006; Buchy et al., 2011; Flashman et al., 2001) and gyrus rectus (Flashman et al., 2001) while awareness of the need for treatment may involve medial frontal, precuneus and inferior temporal cortex (Buchy et al., 2011). On the other hand symptom attribution implicates prefrontal abnormalities including thinness of the DLPFC (Buchy et al., 2012) (Asmal, 2016, in press), thickness of the orbitofrontal cortex (Shad et al., 2006; Buchy et al., 2012), as well as grey matter deficits in the precuneus and posterior cingulate gyrus (Morgan et al., 2010).

Functional MRI (fMRI) studies corroborate structural neuroimaging findings, largely through investigating functional correlates of self-reflective processing (van Buuren et al., 2012; van der Meer et al., 2010a) with limited studies involving a behavioural assessment of insight (van der Meer et al., 2013; Shad and Keshavan, 2015). The difficulty patients have reflecting on the illness, on symptom attribution and on the need for medication may involve self-reflective processing deficits (van der Meer et al. 2010a; van der Meer et al., 2013; Lysaker et al., 2011) mediated by cortical midline structures (CMS), namely the anterior and posterior cingulate cortex, cuneus and precuneus, the ventromedial and dorsomedial prefrontal cortex, and insula (van der Meer et al., 2010b; Ćurčić-Blake et al., 2015; Northoff and Bermpohl, 2004; Murray et al., 2012). Findings from fMRI studies examining brain activation in response to self versus other referential stimuli together with a more direct

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measurement of insight implicate the CMS as well as parietal and temporal cortices (van der Meer et al., 2013; Shad and Keshavan, 2015).

Brain connection irregularities within and between brain regions may be core pathology in schizophrenia (Wheeler and Voineskos, 2014). However, few studies have explored the relationship between white matter tracts and insight in schizophrenia, and none in patients with first episode schizophrenia. Thus far white matter findings in chronic schizophrenia are somewhat conflicting. One study reported no significant association between cognitive insight (cognitive flexibility towards beliefs, judgments and experiences) and DTI measures (FA and mean diffusivity) in 45 people with chronic schizophrenia (Spalletta et al., 2014), while Antonius et al. ($n = 36$) described significant correlations between white matter abnormalities in multiple DTI regions and clinical insight (Antonius et al., 2011). They reported the grey matter region adjacent to the voxels with reduced FA, and found that symptom unawareness was significantly associated with several frontal-temporal lobe regions and symptom misattribution was associated with various parietal and temporal brain regions. Illness chronicity, medication exposure, relatively small sample sizes and the absence of a control group are possible confounders in these chronic samples.

In this study, we performed a DTI analysis of WM tracts in a cohort of first-episode schizophrenia (FES) patients. We were able to avoid potential confounds by selecting a relatively large first-episode sample with minimal or no exposure to antipsychotic treatment and a matched healthy control group. The aim of this study was to identify WM tract differences in FES patients and controls associated with impaired clinical insight. We hypothesized that impaired insight in patients would be predicted by reduced FA values, with a predilection for association tracts interconnecting cortical areas, namely the CMS, and frontal-temporal-occipital areas.

2. Methods

2.1. Participants

This was a substudy of a parent study examining clinical, biological and functional outcome of FES in Cape Town, South Africa. We recruited 125 patients into the parent study; the first 25 were excluded from the neuroimaging component because of an approval delay for scans, 4 were lost as a result of scan error, 3 were unable to be scanned because of claustrophobia, and 4 did not complete the Insight assessment, resulting in a sample of 89 FES patients for the DTI study. We recruited 101 controls and 3 were excluded due to scan error, resulting in 98 controls for the DTI study. First-episode schizophrenia spectrum patients were recruited from inpatient services at Tygerberg and Stikland Hospital, and related community clinics in Cape Town, South Africa. For inclusion in the study, schizophrenia participants had to be aged 16 to 45 years, and experiencing a first psychotic episode meeting Diagnostic and Statistical Manual of Mental Diseases, Fourth Edition, Text Revisions (DSM-IV TR) diagnostic criteria for schizophrenia, schizophreniform or schizoaffective disorder based on the Structured Clinical Interview for DSM-IV (SCID) – Patient Edition (First et al., 2012). The healthy control group was matched for age, sex, ethnicity and level of education (Table 1), and had no DSM-IV axis I or II disorder as determined by the by SCID-Non-Patient Edition interviews. Healthy controls were recruited through personal contacts of the families of the patients with FES and advertisements placed in community centres in the same catchment area as the patients. Patients and controls were excluded if they had a serious or unstable general medical condition, mental retardation, overt substance abuse and <7 completed years of schooling. Patients were excluded if they had a lifetime exposure to >4 weeks of antipsychotic medication or were previously treated with a long-acting depot antipsychotic. Each patient was carefully screened with a thorough physical examination and review of the medical history, ECG, urine toxicology screen and structured assessment of symptoms to verify that inclusion criteria were met. Patients and controls were compensated for transport costs incurred during their participation in the study. Participants did not receive any other financial reward.

This study was conducted according to the principles of the Declaration of Helsinki 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/). After the study procedures were fully explained in accordance with the ethical guidelines of the institutional review board, participants provided written informed consent. The parent study was registered on the South African National Clinical Trials Register (www.sanctr.gov.za/SAClinicalTrials/tabid/169/Default.aspx), trial number DOH-27-0710-1957.

2.2. MRI-acquisition

All scans were acquired with a 3T Siemens Allegra MRI scanner (Erlangen, Germany). Diffusion-weighted images (DWIs) were acquired with the following parameters: $1.8 \times 1.8 \times 2.0$ mm^3 spatial resolution, field of view (FOV) = 220 m, repetition time (TR) = 8800 ms, echo time (TE) = 88 ms, 65 slices, no distance factor and twofold GRAPPA acceleration. The gradients were applied in 30

Table 1
Demographic and clinical characteristics for first-episode schizophrenia patients and matched healthy controls.

Characteristic	Patients ($n = 89$)	Controls ($n = 98$)	Analysis[a]		
			Test statistic	df	p
Age in years (mean, SD)	25.39(6.59)	25.55(7.25)	$t = 1.13$	185	0.13
Male, n (%)	67(75.28)	62(63.27)	$x^2 = 3.15$	1	0.08
Education level, n (%)			$x^2 = 0.72$	3	0.95
Elementary	6(6.74)	8(8.16)			
Secondary	52(58.43)	52(53.06)			
Matriculation	21(23.60)	28(28.57)			
Tertiary	10(11.24)	10(10.20)			
Treatment naïve, n(%)	48(55.81)				NA
Duration of treatment in days (median, [range])	3(0–28)				NA
DUP in days (median, [range])	21.15(1.15–315.29)				NA
BIS (mean, SD)					
Total	6.0(2.19)				
Symptom attribution	2.29(1.05)				
Illness awareness	1.57(1.37)				
Need for treatment	2.14(1.01)				
PANSS G12 (mean, SD)	4.82(1.11)				
MCCB composite score (mean, SD)	16.18(15.76)	29.81(12.69)	$t = 5.88$	152	<0.001

SD, Standard deviation; BIS, Birchwood Insight Scale; DUP, duration of untreated psychosis; PANSS, Positive and Negative Syndrome Scale.
[a] Paired t-tests for continuous variables and x^2 test for categorical variables.

directions and b = 1000 mm/s^2 and 3 b = 0 mm/s^2 images were also acquired. The sequence was repeated three times.

2.3. Image processing

The DWIs were pre-processed using the Functional MRI of the Brain (FMRIB) Software Library (FSL) version 4.1.8 (www.fmrib.ox.ac.uk/fsl/; Smith et al., 2004). Raw DTI data were eddy corrected and the images were imported into Matlab R2008b (Mathworks, MA). The three acquisitions were co-registered by using the first b = 0 mm/s^2 as the reference image. Outliers were determined by calculating the Z-value of the tensor estimates at the 25th and 75th percentiles. Data points falling outside of >3 standard deviations were discarded. The acquisitions were then averaged and exported to the FSL for further processing. Fitting a tensor model to the diffusion-weighted images created fractional anisotropy and mean, axial and radial diffusivity maps. Brain extraction was performed with FSL BET. A study-specific template was created by affine registration of each individual's FA image to the FMRIB58 template, after which images were concatenated and averaged. The template was then affine registered to MNI space and every subject's FA image was non-linearly registered to the template. These transforms were then applied to the original FA images. Masks were created to delineate white matter regions by utilizing the JHU white matter atlas (Mori et al., 2005), with a FA threshold of 0.2 allowing for extraction of each subject's FA image. The mean of the FA per region was extracted and exported to STATA for statistical analysis. The regions of interest are listed in Supplementary Table 1.

2.4. Clinical assessments

Diagnosis and clinical assessment was determined by physicians trained in the use of the key assessment instruments, and inter-rater reliability testing was conducted periodically (intraclass correlation 0.7 or higher). Diagnostic assessment was performed by means of the SCID (First et al., 2012). Insight was measured by means of the Birchwood Insight Scale (BIS) (Birchwood et al., 1994). The BIS assesses clinical insight and comprises eight questions, each scored on a 4-point Likert scale with higher scores indicating better insight. The scale assesses three dimensions of insight: symptom attribution, illness awareness, and need for treatment. The score of the G12 Insight item on the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) was also extracted. Psychotic symptoms were assessed using the complete PANSS (Kay et al., 1987) and factor-analysis derived domains of symptomatology (positive, negative, disorganized, anxiety/depression, excitement) (Wallwork et al., 2012). We assessed depressive symptoms using the Calgary Depression Scale for Schizophrenia (CDSS), cognition with the MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein et al., 2008) and duration of untreated psychosis (DUP) was estimated from the onset of continuous positive psychotic symptoms to initiation of adequate treatment.

2.5. Statistical analyses

Group differences in demographic and clinical characteristics were compared using two sample *t*-tests for continuous variables, and chi-square tests for categorical variables. Histograms were used to assess the normality of distribution of clinical scores and FA values. All tests were 2-tailed and a significance level of 0.05 was used. We corrected for multiple comparisons between patients and controls throughout the analyses using the Benjamini-Hochberg procedure, Simes method (Genovese et al., 2002). We examined for Group X Age, Group X Education and Group X Gender interaction using Pearson Correlation, which yielded no significant interactions. To remove any proxy effects of age, education and gender, residual FA differences between patients and controls were calculated controlling for these measures by a linear regression model. Residuals of the linear regression model were used for further analyses and we used the Least Angle Regression (LARS) algorithm (Efron et al., 2004) to build a prediction model. The "LARS" method is based on a machine learning recursive procedure building a regression model in piecewise linear forward steps. However, LARS produces models with smaller predictive error and has a less severe cost of overfitting than stepwise regression. Hence, it has the advantage of parsimony and prediction accuracy for high dimensional data and predictors that are significantly correlated. LARS uses Mallows Cp (Gilmour, 1996) to address overfitting of the regression model and performs similarly to cross-validation (Efron et al., 2004). Statistical analyses were performed using STATA software v.13 (Stata Corp, 1997).

3. Results

3.1. Participant characteristics

Demographic and clinical characteristics are displayed in Table 1. There were no group differences in age, sex, education and ethnicity. The participants in the FES group scored significantly lower on the MCCB (16.18, sd = 15.76 *v.* 29.81 sd = 12.69). At the time of scanning, all FES participants were either minimally treated (n = 40, 45%) in that they were exposed to antipsychotic treatment for a short period prior to recruitment into the study (median duration of treatment 3 [range1–28] days, or antipsychotic naïve (n = 49, 55%)).

3.2. Group differences in FA values

FA values were significantly reduced bilaterally in most of the tracts in FES patients compared to controls after correction for multiple comparisons (Supplementary Table 1, q < 0.05). There were no significant increases in FA in patients compared to controls. Subgroup analysis demonstrated that there were no significant differences in FA in any tract between minimally treated and treatment naïve patients (p > 0.05).

3.3. Relationship between clinical variables and insight

Results are presented in Table 2. Greater depressive symptoms as measured by the Calgary Depressive Symptom Scale and by the PANSS Depression subscale were associated with higher BIS score (indicating better insight). There were no significant correlations between insight and PANSS total or subscale scores, or duration of untreated psychosis. Cognitive data available in a subsample of the FES participants (n = 60) revealed no association between insight and MCCB composite scores.

3.4. Insight and FA values

Residual FA values of white matter tracts, together with clinical factors associated with insight (depression as measured by the CDSS) were entered into the LARS prediction model for insight. Tracts predicting insight remained unchanged whether residuals or unadjusted FA were used, however multicollinearity was considerably reduced by the use of residual values (variance inflation factor = 4) as opposed to unadjusted FA values (variance inflation factor = 8). We found that FA values in a network of tracts predicted insight (Table 3, Fig. 1). The complex network of tracts and the direction of the relationship between FA values and global insight is summarized as follows: cingulum (RH+, LH−); cingulate hippocampus (LH+); uncinate faciculus (RH+, LH−); superior fronto-occipital fasciculus (RH+), anterior (RH+) and posterior (LH−) corona radiata, external capsule (RH+), inferior (LH−) and superior (RH+) cerebellar peduncle, posterior thalamic radiation (LH−), corticospinal tract (RH+), and splenium of the corpus callosum (+). We also found that lower CDSS scores for depression (more severe

Table 2
Association between total insight and clinical characteristics in patients.

Characteristic	Mean [SD/range]	Total insight[a]	Symptom attribution[a]	Illness awareness[a]	Need for treatment[a]
DUP in weeks (median, [range])	21.15 [1.15–315.29]	0.125	0.041	0.085	0.125
PANSS scores (mean, SD)					
Total	92.66 (15.28)	0.029	0.018	−0.027	0.081
Negative factor	16.94(4.42)	−0.059	0.049	−0.162	0.041
Positive factor	13.46(2.74)	0.027	−0.027	0.098	−0.046
Excitement factor	7.98(3.58)	0.012	0.039	−0.067	0.075
Disorganized factor	17.75(4.35)	−0.172	−0.065	−0.091	−0.181
Depressive factor	9.04(4.10)	0.324*	0.041	0.236*	0.302*
CDSS (mean, range)	3.15 (0–17)	0.412*	0.055	0.344*	0.341*
MCCB composite (mean, range)	16.18(15.76)	0.162	0.178	−0.028	0.205

SD, Standard deviation; BIS, Birchwood Insight Scale; DUP, duration of untreated psychosis; PANSS, Positive and Negative Syndrome Scale; CDSS, Calgary Depression Rating Scale for Schizophrenia.

[a] Pairwise correlation for bivariate normal and spearman's correlation for nonparametric variables.

* $p < 0.01$.

depressive symptoms) predicted poorer insight. In a sensitivity analysis of unadjusted FA values, we explored whether gender had a significant impact on insight and we found no association. We performed exploratory analyses using the LARS algorithm looking at predictors of lower scores on the BIS subscales. We found that fewer WM tracts predicted 'awareness of illness' and 'need for treatment' compared to global insight, and that the networks of tracts predicting these subdomains were separable. FA values did not predict 'symptom attribution'. 'Awareness of illness' was predicted by cingulum (LH−), cingulum hippocampus (LH+), sagittal stratum (LH+), superior cerebellar peduncle (LH+). 'Need for treatment' was predicted by splenium of corpus callosum (−), superior fronto-occipital fasciculus (RH-), uncinate fasciculus (LH−), anterior corona radiata (LH+).

4. Discussion

Here we investigated associations between white matter FA and insight in the largest DTI cohort of first-episode schizophrenia patients to date. We found widespread reduction in FA in FES patients compared to controls. Importantly, our findings are in keeping with that of structural MRI, fMRI and limited DTI findings suggesting that the neural signature of insight is widespread (Buchy et al., 2011) with a predilection for those tracts associated with cortical midline structures. Our results also suggest that the association between FA changes and insight was most robust for the awareness of symptoms and illness awareness domains of insight.

Table 3
Prediction model of insight.

White matter tracts	Global insight	Illness awareness	Need for treatment
Adjusted R^2	0.35	0.25	0.17
β coefficient			
Splenium corpus callosum	8.33		3.05
Sup FOF: RH	−8.35		−3.53
Cingulum: RH	0.69		
LH	−15.35	−16.23	
Cingulum hpc: LH	8.21	5.04	
Uncinate fasciculus: RH	5.20		
LH	−4.06		−4.80
External capsule: RH	2.93		
Sagittal stratum: LH	6.16	7.00	
Post corona radiata RH	−27.00		
Ant corona radiata: LH	3.68		2.83
Sup cerebellar ped: LH	12.31	2.36	
Inf cerebellar ped: RH	−11.07		
Post thalamic radiation: RH	−5.60		
Corticospinal tract: RH	3.69		
CDSS total score	0.11	0.076	

Key: RH = right hemisphere, LH = left hemisphere; sup FOF = superior frontooccipital fasciculus; cingulum hpc = cingulum hippocampus; ant = anterior; post = posterior; inf = inferior; cerebellar ped = cerebellar peduncle.

Poor insight has consistently been associated with anterior and posterior cortical midline structures namely medial frontal, anterior and posterior cingulate, cuneus and precuneus, and inferior temporal brain regions (Ćurčić-Blake et al., 2015). An association between insight deficits and abnormalities within the CMS is largely based on structural and fMRI studies and our results extend these findings by highlighting abnormalities in anterior CMS related tracts namely cingulate, cingulate hippocampus, uncinate, superior fronto-occipital, and anterior corona radiata, and with tracts related to posterior CMS structures, namely posterior corona radiata and splenium of the corpus callosum. This is in keeping with the anosognosia model of insight where core deficits in the dorsolateral prefrontal cortex, anterior cingulate cortex, orbitofrontal cortex and parietal cortex are proposed to mediate aberrant self-monitoring in symptom unawareness, and communication deficits between these regions possibly account for poor insight (Shad et al., 2007).

Functional neuroimaging studies implicate involvement of CMS largely through self-reflective processing tasks (van der Meer et al. 2010a; Ćurčić-Blake et al., 2015). Self-reflection involves the ability to accurately evaluate one's own actions, thoughts and attitudes and compare it with that of others and poor self-reflective ability may be central to impairments in insight. (van der Meer et al. 2010a; van der Meer et al., 2013; Lysaker et al., 2011). One model of self-reflection hypothesizes that different CMS regions are involved in different functions within a self-reflective processing pathway such as self-directed attention, affective processing of information, monitoring and evaluation (van der Meer et al. 2010a; van der Meer et al., 2013; Lysaker et al., 2011). Failure of integrated action as a result of abnormal or dysfunctional connectivity may underlie core deficits in schizophrenia (Stephan et al., 2009) and insight and self-reflection are complex processes that would be expected to rely on the integrated action of several higher cortical regions and the CMS (Ćurčić-Blake et al., 2015).

Our findings also provide preliminary evidence for the existence of separable tracts involved in the different domains of insight. In our prediction model, tracts centered on the CMS were associated with the domains of Need for Treatment and Awareness of Illness, while Symptom Misattribution was not related to WM tract abnormalities. Illness awareness involves recognition of signs and symptoms, while attribution concerns the explanation a person has of the source and cause of these signs and symptoms (Amador et al., 1994). The WM changes associated with symptom unawareness in this study are consistent with those found in a previous DTI study in chronic schizophrenia (Antonius et al., 2011). In that study, the authors also found that while symptom unawareness was associated with a number of fronto-temporal white matter deficits, the association between lower FA and

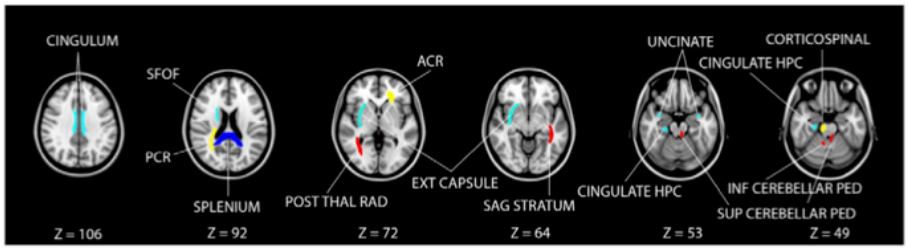

Fig. 1. White matter tracts predicting insight. Figure legend: A network of afferent projection (red), efferent projection (yellow), association (light blue), and commissural (dark blue) tracts predicted poorer insight. Our results highlight the involvement of the cingulum, cingulate hippocampus (cingulate HPC), uncinate faciculus, superior fronto-occipital fasiculus (SFOF), anterior and posterior corona radiata (ACR, PCR), external capsule, inferior and superior cerebellar peduncle, posterior thalamic radiation, corticospinal tract, and splenium of the corpus callosum.

symptom misattribution was localized to fewer white matter areas (near the precuneus, lentiform nucleus and middle temporal gyrus). In keeping with this finding, more recent functional MRI studies suggest that somewhat distinct neurobiological dysfunction may subserve deficits in each insight dimension. In contrast to the widespread network of brain areas implicated in other insight domains, a recent functional neuroimaging study also describes fewer and more specific frontal areas involved in symptom misattribution, which may indicate a failure to recruit compensatory brain mechanisms (Shad and Keshavan, 2015). This may lend support to the theory that symptom unawareness is a reflection of a more complex brain network disorder involving a number of brain regions, while symptom misattribution may be mediated by deficits in fewer and more specific brain regions (Shad and Keshavan, 2015). Our negative finding may therefore in part be explained by the possibility that the fewer tracts proposed to be related to symptom misattribution may not having survived statistical modeling in our study.

Our predictive model also implicates other WM fibre tracts being related to insight, including the involvement of thalamic and cerebellar connections. This is not unexpected since an increasing number of neuroimaging studies have shown that the thalamus is involved in integrative cognitive processing, relaying not only sensory information, but also regulating information transmitted to frontal and higher order sensory cortices according to cognitive demands and dynamically routing information across the brain (Saalmann et al., 2012; Saalmann and Kastner, 2015). The traditional view that the cerebellum is simply involved in movement has also been increasingly challenged (Middleton and Strick, 2000). Cerebellar grey matter deficits have been significantly correlated with insight impairment (Bergé et al., 2011) and fMRI studies have described cerebellar activation and symptom unawareness (Shad and Keshavan, 2015) and emotional unawareness (alexithymia) (Moriguchi et al., 2007).

In our study, patients with greater insight were more depressed. Depressive symptoms were significantly associated with greater global insight, increased awareness of the illness and the recognition of the need for treatment, in both the correlation analysis and in the prediction model. Meta-analyses of studies using the BIS as an insight measure, have found that depression is significantly associated with global insight (Mintz et al., 2003; Murri et al., 2015), insight into illness (Mintz et al., 2003; Murri et al., 2015) and need for treatment (Mintz et al., 2003). The relationship between insight and depression is proposed to be bidirectional, particularly in the acute phase (the emergence of depression as insight is acquired on the one hand and poor insight as a form of denial in order to ward-off depressive symptoms on the other hand) (Murri et al., 2015). While, the direction of causality as well as the nature of underlying neurobiological pathways remains unclear, our data highlight the possible involvement of white matter tracts.

We did not find a significant relationship between insight and other clinical variables; in keeping with meta-analyses suggesting that global, positive and negative symptomatology account for a small proportion of the variance in insight in schizophrenia (Mintz et al., 2003). Similarly, regarding cognitive dysfunction, while some report a specific association between insight and executive dysfunction (Young et al., 1998; Young et al., 2003a) and others describe an association with more generalized cognitive deficits (Keshavan et al., 2004), particularly in FES samples the relationship between cognition and insight is modest and inconsistent, (David et al., 2012). Tracts predicting insight in our study, particularly those associated with the fronto-temporal areas are also associated with clinical symptoms in other schizophrenia studies. For example higher FA in the cingulum bundle (Seok et al., 2007; Cheung et al., 2011; Fujiwara et al., 2007) and lower FA in the left uncinate fasciculus (Skelly et al., 2008) and left cerebellar tracts (Zhang et al., 2016) may be associated with positive symptoms. Lower FA in the uncinate fasciculus has also been associated with greater severity of negative symptoms (Szeszko et al., 2008), while corpus collosum FA (uncorrected) has been significantly positively correlated with general symptoms (Zhang et al., 2016). FA alterations in tracts predictive of insight were also significantly associated with neurocognitive performance in other studies. For example, left thalamic FA has been significantly correlated with spatial working memory deficits (Qiu et al., 2009), and lower FA in the uncinate fasciculus has been correlated significantly with worse verbal learning and memory (Szeszko et al., 2008; Nestor et al., 2008), while lower FA in the cingulum bundle has been associated with executive function and intelligence (Nestor et al., 2008).

Finally, we found decreased FA in minimally treated FES patients compared to controls across 41 of the 48 WM tracts examined, in both hemispheres, after controlling for multiple comparisons. Our findings lend considerable support to the idea that brain abnormalities in schizophrenia as measured by DTI are widespread (Wheeler and Voineskos, 2014) and substantiate reviews describing significant reduction in FA in frontal and temporal areas, and abnormalities within the fibre bundles interconnecting these regions as among the most frequent positive findings (Wheeler and Voineskos, 2014; Pettersson-Yeo et al., 2011; Ellison-Wright et al., 2008). Importantly, our subgroup analysis revealed no significant differences in FA between minimally treated and treatment naïve patients but clear differences between patients and controls, suggesting that these changes are likely disease related and present prior to treatment with antipsychotic medication.

Strengths of our study include the large sample, the selection of first-episode, minimally treated patients, inclusion of matched controls from the same community as the FES patients, and the use of a validated instrument to assess insight. Several limitations should be noted. The BIS is a self-rated instrument. This may be regarded as a limitation in that a researcher-rated instrument may plausibly provide a more objective measure of insight. On the other hand self-report questionnaires may eliminate potential researcher and clinician biases (Young et

al., 2003b). Also, we hypothesized that there is involvement of a number of DTI tracts in insight deficits in schizophrenia and therefore examined a relatively large number of potential predictor variables. Poor insight has been linked to neurocognitive deficits, and although we did perform cognitive assessments on a smaller sample of the cohort, we did not assess IQ. Although our study is larger than many other DTI studies, statistical power is limited when a large number of variables are included. We attempted to mitigate this limitation by using the LARS statistical method that was developed to select a parsimonious set of prediction variables compared to traditional forward selection models.

In conclusion, this study provides evidence for involvement of widespread white matter tracts in insight deficits in schizophrenia, in particular those associated with CMS (namely fronto-occipital, cingulate, cingulate hippocampus, uncinate, anterior corona radiata). Insight is a complex entity and in this study we used the BIS to focus on clinical insight. Further works will require exploration of other constructs of insight, such as cognitive insight; clinician rated insight, as well as related insight domains. In future studies, fibre tractography would be helpful to further distinguish the neural networks implicated in insight and insight domains in schizophrenia in a more directed manner. Even so, this is a first step in delineating the white matter tracts involved in insight impairment in schizophrenia prior to chronicity.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2016.11.005.

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Supplementary Table 1: Fractional anisotropy (FA) differences between patient and healthy control groups after correction for multiple comparisons

Fibre tract	Right hemisphere			Left hemisphere		
	Mean difference	95% CI	p-value	Mean difference	96% CI	p-value
Association fibres						
Cingulum	-0.021	-0.033 to -0.010	<0.001	-0.011	-0.018 to -0.003	0.004
Cingulum hpc	-0.007	-0.015 to 0.001	0.058	-0.015	-0.026 to -0.003	0.017
Sup Long Fasc	-0.001	-0.014 to 0.012	0.882	-0.009	-0.019 to 0.002	0.11
Sup Fronto-Occipital Fasc	-0.007	-0.016 to 0.001	0.069	-0.017	0.027 to -0.006	0.104
Uncinate fasc	-0.014	-0.031 to 0.002	0.087	-0.017	-0.029 to -0.005	0.003
Sagittal stratum	-0.017	-0.028 to -0.006	0.003	-0.017	-0.028 to -0.006	0.002
External capsule	-0.019	-0.030 to -0.009	<0.001	-0.021	-0.035 to -0.007	0.004
Fornix	-0.013	-0.02 to -0.03				0.031
Stria terminalis	-0.013	-0.026 to 0.001	0.07	0.001	-0.011 to 0.011	0.94
Commisural fibres						
Corpus callosum genu	-0.021	-0.032 to -0.010	<0.001		NA	
Corpus callosum body	-0.022	-0.033 to -0.012	<0.001		NA	
Corpus callosum splenium	-0.012	-0.023 to -0.001	0.032		NA	
Tapetum	-0.016	-0.027 to -0.005	0.003	-0.011	-0.022 to 0.001	0.031
Afferent projection fibres						
Sup cerebellar ped	-0.010	-0.017 to -0.002	0.007	-0.012	-0.022 to -0.002	0.015
Middle cerebellar ped		NS		-0.016	-0.025 to -0.006	0.001
Pontine crossing tract:						
Inf cerebellar ped	-0.010	-0.017 to -0.003	0.007	-0.014	-0.024 to -0.004	0.015
Int capsule: ant limb	-0.009	-0.019 to -0.001	0.047	-0.011	-0.019 to -0.004	0.002
Int capsule: post limb	-0.012	-0.020 to -0.005	0.002	-0.012	-0.020 to -0.004	0.004
Int capsule: retrolenticular	-0.013	-0.021 to -0.004	0.005	-0.012	-0.022 to -0.002	0.002

Post thalamic radiation	-0.123	-0.243 to 0.000	0.045	-0.017	-0.030 to -0.004	0.009
Medial lemniscus	-0.012	-0.021 to -0.003	0.009	0.015	-0.025 to -0.006	0.002
Efferent projection fibres						
Ant corona radiata:	-0.017	-0.028 to -0.006	0.003	-0.016	-0.028 to -0.006	0.002
Sup corona radiata	-0.011	-0.022 to -0.001	0.029	-0.011	-0.020 to -0.001	0.024
Post corona radiata	-0.006	-0.012 to 0.000	0.051	-0.007	-0.132 to -0.001	0.032
Cerebral peduncle	-0.014	-0.023 to -0.004	0.007	-0.013	-0.023 to -0.004	0.006
Corticospinal tract	-0.013	-0.023 to -0.004	0.0008	-0.014	-0.023 to -0.006	0.001

P-value adjusted for multiple comparison: p=0.03

CHAPTER 3:

SYMPTOM ATTRIBUTION AND FRONTAL CORTICAL THICKNESS IN FIRST-EPISODE SCHIZOPHRENIA

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Original Article

Symptom attribution and frontal cortical thickness in first-episode schizophrenia

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Abstract

Aim: Misattribution of symptoms is a common feature of schizophrenia, and likely involves impairment of metacognitive function that may be mediated by the frontal cortex. We aimed to compare frontal cortical thickness in first-episode schizophrenia (FES) patients with matched controls, and investigate its relationship with the symptom attribution dimension of insight in FES patients.

Methods: We examined frontal cortical thickness in 92 minimally treated FES patients at baseline presentation and 93 healthy controls aged 16–45 years. We examined for correlations between symptom attribution as determined by the Birchwood Insight Scale (BIS) symptom relabeling subscale score and cortical thickness of frontal regions of interest (ROIs). We then examined for an association between symptom attribution and cortical thickness using multiple regression analysis.

Results: FES patients exhibited significantly reduced cortical thicknesses for a number of frontal regions, namely the left medial orbitofrontal, left superior frontal, left frontal pole, right rostral middle frontal, right lateral orbitofrontal and right superior frontal regions. Reduced cortical thickness in FES patients was associated with symptom misattribution for the left and right rostral middle frontal, left caudal anterior cingulate, right superior frontal, and left and right pars triangularis regions. Reduced left rostral middle frontal thickness and left anterior cingulate thickness remained significant on regression analysis.

Conclusion: Our findings suggest that frontal neuroanatomical deficits that are present early in the disease process may be critical to the pathogenesis of symptom attribution in schizophrenia.

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INTRODUCTION

Impaired insight is a highly prevalent feature of schizophrenia that is associated with poor outcome in several domains, including increased severity of illness,[1] poor treatment adherence,[2] symptom recurrence,[3] increased use of services,[4] poor psychosocial functioning[5] and poorer prognosis.[6] Over the years, our conceptualization of insight has evolved from a binary construct (patients either lack or have insight) to a more complex multidimensional entity. The clinical model details three subcomponents of insight[7]: awareness of a mental disorder, compliance with treatment and the ability to detect and label mental events as pathological (termed symptom attribution).

While illness awareness involves recognition of signs and symptoms, attribution concerns the explanation a person has of the source and cause of these signs and symptoms.[8] Symptom attribution subsumes a degree of symptom awareness, in that awareness of symptoms is required to enable appropriate attribution.[9] However, relabeling signs and symptoms as pathological involves a higher level of insight function, and David and co-workers[9] argue that symptom attribution is metacognitive in nature in that it involves the ability to monitor and control ongoing mental activities.

Based on a meta-analysis of neuropsychological functioning and insight in psychosis, the ability to recognize and attribute symptoms is also hypothesized to be the insight domain most strongly associated with poor neurocognitive function.[9] The frontal cortex is involved in information synthesis, concept formation, mental flexibility, self-awareness and self-monitoring,[7,10] and findings from neurocognitive and neuroimaging data suggest that insight deficits may be related to the integrity of the prefrontal cortex.[9,11,12] Furthermore, in healthy subjects, the ventral medial prefrontal cortex and dorsal medial prefrontal cortex are key areas implicated in self-referential processing believed to be fundamental to the default-mode network (DMN) (brain areas with increased activation at rest compared with when doing a task).[13] There is some evidence that in patients with schizophrenia and their first-degree relatives, aberrant connectivity within the DMN may contribute to associated self-referential processing difficulties.[14]

In keeping with the conceptual differentiation between insight domains, results from structural and more recently functional magnetic resonance imaging (fMRI) studies suggest that somewhat distinct neurobiological dysfunction may subserve deficits in each insight dimension. In contrast to the widespread network of brain areas implicated in other insight domains, functional neuroimaging studies describe fewer and more specific frontal areas involved in symptom misattribution, which may indicate a failure to recruit compensatory brain mechanisms.[15,16]

However, few neuroimaging studies have examined the relationships between grey matter volume/thickness and symptom attribution in schizophrenia. An early study by Flashman *et al.*[17] examined the relationship between symptom misattribution and brain volume across eight frontal lobe subregions in 15 patients with chronic schizophrenia and found an inverse relationship between symptom misattribution and bilateral superior frontal gyrus volume. In a region of interest (ROI) study of 14 first-episode antipsychotic-naive schizophrenic patients and 21 controls, Shad *et al.*[18] described an association between increased right medial orbitofrontal cortex (OFC) volume and symptom misattribution in first-episode schizophrenia (FES) patients. Buchy *et al.* assessed symptom attribution and cortical thickness across the entire cerebrum in 52 patients with first-episode psychosis.[19] With regards to the frontal lobe, the authors described associations between symptom misattribution and cortical thinness in the left middle frontal gyrus and increased cortical thickness in the medial OFC and delusional misattribution. However, the authors described their study as exploratory in that an analysis across the entire cerebrum has less statistical power than a predefined ROI approach due to the number of multiple comparisons, and also recommended that future studies include a control group.

While the ability to attribute symptoms as pathological is dependent on having awareness of the symptoms, structural magnetic resonance imaging (MRI) data thus far suggest that these insight dimensions have related but different neurological substrates.[9] Symptom attribution is a relatively understudied dimension of insight and to extend the findings of the studies described above, here, we examine cortical thickness in frontal ROIs in a large sample of antipsychotic-naive or minimally treated FES patients (n = 92) and matched controls (n = 93), and investigate its relationship with the symptom attribution dimension of insight in FES patients. We hypothesized that frontal cortical thickness is decreased in FES patients compared with controls, and that there is an association between frontal cortical thickness and attribution of symptoms in FES patients.

METHODS

Participants

The study comprised a cross-sectional assessment of 92 patients with FES and 93 healthy controls recruited through the First-Episode Schizophrenia Research Unit at Stellenbosch University, Cape Town, South Africa. All patients were scanned and clinically assessed in the acute stage of illness (including assessment of insight), within 1 week of recruitment into the study. Inclusion criteria for patients and controls were male or female subjects aged 16–45; and a negative history of current substance abuse (as confirmed by a urine drug screen) or a serious medical condition. Following comprehensive history-taking by a psychiatrist, we excluded controls with a first-degree family member with a psychotic illness, and patients and controls with a history of significant head injury. All patients and controls were assessed by a medical doctor who screened for medical conditions that were judged as serious enough to impact assessment or to present as psychiatric illness. Patients were excluded if they had, during their lifetime, been exposed to >4 weeks of antipsychotic medication or had been treated with a long-acting depot antipsychotic or were intellectually disabled (IQ < 70). Patient diagnosis of either schizophrenia, schizophreniform disorder or schizoaffective disorder was based on the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental

reliability criteria in acute schizophrenia and displays convergent validity with measures obtained using a semi-structured interview.[29,30] The BIS comprises three subscales that assess different insight dimensions: symptom attribution, illness awareness and need for treatment. Each question of the BIS is rated 0–2 and each dimension is rated on a scale of 0–4. Higher total scores indicate better insight. The symptom attribution dimension comprises two questions: 'some of the symptoms were made by my mind' and 'none of the unusual things I experienced are due to an illness'. Each question can be marked as agree, disagree or unsure by the participant. Severity of clinical symptoms of schizophrenia was assessed by the Positive and Negative Syndrome Scale (PANSS) total score[31] and depressive symptoms using the Calgary Depression Scale for Schizophrenia (CDSS),[32] and duration of untreated psychosis was estimated from the onset of continuous positive psychotic symptoms to initiation of adequate treatment. Investigators were physicians trained in the use of the PANSS, and inter-rater reliability testing was conducted periodically (intraclass correlation of 0.7 or higher).

Analyses

To compare demographic characteristics between groups, we used two sample *t*-tests for continuous variables and chi-square test for categorical variables. Data were examined for normality using the skewness and kurtosis test, and non-parametric tests (rank transformation) were used for data that were not normally distributed. For both hemispheres, we selected all FreeSurfer ROIs for the frontal lobe as per Desikan *et al.*'s[23] automated labelling system. These are the superior frontal gyrus, rostral and caudal divisions of the middle frontal gyrus, pars opercularis, pars triangularis, pars orbitalis, lateral and medial divisions of the OFC, frontal pole, precentral gyrus and paracentral lobule. We corrected for multiple comparisons using false discovery rate and Simes' method.[33] Residual cortical thickness differences between FES patients and controls were calculated controlling for age and gender by a linear regression model. Residuals of the linear regression model were used for further analyses. We conducted two-sample *t*-tests comparing age- and gender-adjusted frontal cortical ROI thickness differences between FES patients and healthy controls (recalibrated threshold of significance after correction for multiple comparisons, $P = 0.01$). Pairwise correlations were performed between the BIS 'symptom attribution' subscale score (normally distributed) and cortical thickness of frontal ROIs (recalibrated threshold of significance after correction for multiple comparisons, $P = 0.01$). Meta-analyses describe a small but consistent negative relationship between insight and positive and negative symptoms, and a small but consistent negative relationship between insight and depression.[1,34] We then examined the association between symptom attribution and cortical thickness using multiple regression analysis and adjusted for PANSS negative and positive factors and CDSS.

RESULTS

Demographic and clinical characteristics

Demographic and clinical characteristics are provided in Tables 1 and 2. Age, gender, education level and total intracranial volume did not differ between FES patients and controls. Patients were treatment naive ($n = 54$) or minimally treated (median (range) duration of treatment = 3 (0–28) days) ($n = 38$) at the time of assessment. There was no difference in age ($P = 0.95$), gender ($P = 0.35$), PANSS total ($P = 0.10$) or intracranial volume ($P = 0.25$) between treatment-naive and minimally treated FES patients. The BIS total score correlated significantly with the PANSS G12 score ($r = -0.336$, $P = 0.001$) suggesting significant convergent validity of the BIS. Symptom misattribution did not correlate significantly with severity of psychosis, depression or with functional impairment (Table 2).

TABLE 2. Association between symptom attribution and clinical characteristics in FES patients

Characteristic	Mean (SD/range)	Correlation coefficient†	*P*-value‡
DUP in weeks (median, range)	21.15 (1.15–315.29)	0.012	0.96
PANSS scores (mean, SD)			
Total	92.66 (15.28)	0.021	0.84
Negative factor	16.98 (4.58)	0.047	0.65
Depressive factor	8.93 (4.04)	0.056	0.59
Positive factor	13.47 (2.75)	−0.053	0.62
Excitement/ hostility factor	8.04 (3.69)	0.05	0.61
Disorganized factor	17.75 (4.35)	−0.07	0.50
CDSS (mean, range)	2.98 (0–17)	0.092	0.38
SOFAS		−0.11	0.26
CGI		−0.01	0.89

†Pairwise correlation for bivariate normally distributed and Spearman's correlation for non-parametric variables.
‡Paired *t*-tests for continuous variables and chi-square test for categorical variables.
CDSS, Calgary Depression Scale for Schizophrenia; DUP, duration of untreated psychosis; FES, first-episode schizophrenia; PANSS, Positive and Negative Syndrome Scale; SD, standard deviation.

Differences in prefrontal cortical thickness between FES patients and controls

Cortical thickness was reduced in FES patients compared with matched controls in the left medial orbitofrontal, left superior frontal, left frontal pole, right rostral middle frontal, right lateral orbitofrontal and right superior frontal regions after correcting for multiple comparisons (recalibrated threshold of significance, $P = 0.01$; Table S1, Supporting information). Subgroup analysis demonstrated that there was no significant difference in cortical thickness in any frontal region between minimally treated and treatment-naive FES patients.

Associations between symptom attribution and cortical thickness

Average cortical thickness for the following regions was significantly correlated with the ability to relabel symptoms as pathological after adjusting for age and gender (corrected for multiple comparisons): left and right rostral middle frontal (left: $r = 0.305$, $P < 0.01$; right: $r = 0.305$, $P < 0.01$), left and right pars triangularis (left: $r = 0.241$, $P = 0.02$; right: $r = 0.257$, $P = 0.01$), left caudal anterior cingulate ($r = 0.243$, $P = 0.02$) and right superior frontal ($r = 0.240$, $P = 0.02$) (Table 3). Stepwise linear regression was used to identify variables that were independently associated with symptom attribution. The regression model showed that thinness of the left rostral middle frontal region ($\beta = 1.48$, 95% confidence interval (CI) = 0.45–2.51, $P < 0.01$) and left caudal anterior cingulate ($\beta = 0.87$, 95% CI = 0.07–1.68, $P = 0.03$) was associated with poor symptom attribution (Table 4). Although not significant on bivariate analysis, PANSS negative and positive factors and CDSS total score were included in the multivariate model because they are shown in meta-analytic studies to have small but consistent associations with insight in schizophrenia. None of these clinical symptoms were significantly associated with symptom attribution on the regression model.

TABLE 3. Association between symptom attribution and cortical thickness of FreeSurfer predefined frontal ROI's (critical threshold $P = 0.02$, after correction for multiple comparisons)

Frontal region	Correlation coefficient	*P*-value
Left hemisphere		
Rostral middle frontal	0.305	0.01
Caudal middle frontal	0.146	0.16
Rostral anterior cingulate	0.204	0.05
Caudal anterior cingulate	0.243	0.02
Lateral orbitofrontal	0.150	0.15
Medial orbitofrontal	0.128	0.22
Pars opercularis	0.194	0.06
Pars orbitalis	0.120	0.25
Pars triangularis	0.241	0.02
Precentral	0.027	0.80
Superior frontal	0.220	0.03
Frontal pole	0.168	0.11
Right hemisphere		
Rostral middle frontal	0.305	0.01
Caudal middle frontal	0.118	0.26
Rostral anterior cingulate	0.137	0.19
Caudal anterior cingulate	0.065	0.54
Lateral orbitofrontal	0.139	0.19
Medial orbitofrontal	0.035	0.74
Pars opercularis	0.142	0.18
Pars orbitalis	0.182	0.08
Pars triangularis	0.257	0.01
Precentral	0.119	0.26
Superior frontal	0.240	0.02
Frontal pole	0.150	0.15

ROI, region of interest.

DISCUSSION

In this study, we compared frontal cortical thickness in minimally treated FES patients to matched healthy controls. In FES patients, we investigated the association between symptom attribution deficits and cortical thickness in frontal areas. First, our results show a reduction in cortical thickness in minimally treated FES patients compared with controls across several frontal regions in both hemispheres, confirming results of previous volumetric[34–36] and more recent cortical thickness FES studies.[37,38] In keeping with the findings of Xiao *et al.* who described prominent and widespread decreased cortical thickness in frontal regions in neuroleptic-naive FES patients compared with controls,[39] our subgroup analysis revealed no significant differences in cortical thicknesses of frontal ROIs between minimally treated and treatment-naive FES patients but clear differences were observed between FES patients and controls, suggesting that anatomical changes are likely disease related and present prior to treatment with antipsychotic medication.

Second, we found that lower misattribution scores correlated with a thinner cortex for a number of frontal regions (left and right rostral middle frontal, left caudal anterior cingulate, left and right pars triangularis and right superior frontal). Of these regions, the right rostral middle frontal and right superior frontal also displayed decreased cortical thickness in FES patients compared with controls. On regression modelling, reduced left rostral middle frontal thickness and left anterior cingulate thickness accounted for 13.8% of the variance in symptom attribution.

TABLE 4. Regression model of symptom attribution

Explanatory variable	Model parameters			Overall model summary	
	β	*P*	95% CI	R^2	*P*
				0.138	<0.01
Left rostral middle frontal thickness	1.48	<0.01	0.45–2.51		
Left anterior cingulate thickness	0.87	0.03	0.07–1.68		

CI, confidence interval.

By focusing on the frontal lobes, our findings considerably strengthen that of a prior whole-brain cortical thickness study[19] that also implicated a number of frontal lobe areas in symptom misattribution, namely the precentral gyrus, OFC, superior middle and inferior frontal gyri and medial frontal gyrus. Furthermore, unlike the negative findings from insight studies examining grey matter volume in FES,[10,19] the observed reduction in bilateral frontal cortical thickness further supports the notion that cortical thickness may be a more sensitive measure of cortical changes than volumetric studies.[38] Rightward lateralization of reduced prefrontal volume has been described in some studies of schizophrenia patients with impaired insight suggesting an association between insight deficits in schizophrenia and anosognosic syndromes found in neurological patients.[12,39] In contrast, although we did find evidence of the proposed involvement of non-dominant frontal regions, the association between symptom attribution and cortical thinness was widespread in both hemispheres.

Our finding of a robust association between anterior cingulate cortical thickness and symptom misattribution lends support to Shad *et al.*'s theory that the anterior cingulate cortex may be key factor involved in decision-making and salience attribution. They argue that inability to integrate sensory information and related aberrant salience attribution to these stimuli may contribute to pathological symptom misattribution in schizophrenia. Our results also strengthen the findings of other studies implicating the dorsolateral prefrontal cortex (DLPFC) in symptom attribution in schizophrenia.[12,17,19,39,40] The DLPFC, akin to the rostral middle frontal region,[41] plays a crucial role in executive functioning, concept flexibility and self-monitoring. These cognitive abilities are central to the ability to appropriately relabel symptoms. Our findings also corroborate an earlier study by Flashman *et al.*[17] who described an association between smaller superior frontal gyrus volume and greater symptom misattribution. Intriguingly, an fMRI study investigating brain activity engaged during self-reflective introspection describes activated regions in the superior frontal gyrus extending to the anterior cingulate as well as bilateral activation of the inferior frontal gyrus (which subsumes the pars triangularis). This is in keeping with the findings of our study and supports the hypothesis that symptom attribution is metacognitive in nature.[42]

Although it is argued that distinct neurobiological substrates underlie the different insight dimensions,[9] there may be shared neuroanatomical and clinical elements between the dimensions. For example, an awareness of symptoms may impact a patient's belief in need for treatment and on the attribution of symptoms. The DLPFC has been implicated in both awareness of illness and, more recently, symptom attribution deficits in FES.[9,12,19,40] Indeed, various evolving models of insight using structural and fMRI and cognitive data have been suggested to integrate the relationship between symptom attribution, need for treatment and symptom awareness.[9,42]

Our study has several strengths. The use of a comparatively large group of carefully clinically characterized minimally treated FES patients matched with healthy control subjects minimized the potential effects of chronic illness, medication and sociodemographic variables on brain structure. We used a conservative correction to minimize false-positive results from multiple comparisons in the cortical thickness and correlation analyses. There were several limitations. Many FES patients were exposed to small quantities of antipsychotics, so the FES sample cannot be regarded as treatment naive. Data is cross-sectional and it is not possible to draw conclusions about the significance of our findings in relation to the course of illness. We did not find a significant difference in intracranial volume between FES patients and controls, in contrast to meta-analyses that describe a small but significant reduction in intracranial volume in schizophrenia patients compared with controls.[43] Although we matched FES patients and controls for gender, there may still be differential proxy effects of male gender on intracranial volume that we did not account for. Another potential reason is that intracranial volume may be a reflection of early neurodevelopmental processes; white matter loss has been found to be equivalent to the decrease in intracranial volume in FES patients[43] and future planned studies based on our data will look at white matter. Given that our understanding of insight has evolved into a complex multifaceted entity, it is likely that a network of brain areas underpins the various dimensions of impaired insight. Other areas such as the temporal and parietal lobe and regions therein

are implicated in symptom misattribution. We chose to elucidate the relationship between frontal regions and symptom misattribution because this association is consistently described but studies thus far are limited either by a large number of comparisons (as in exploratory whole-brain analysis), illness chronicity or small sample sizes.

In conclusion, these findings further strengthen our understanding of the neurobiological basis of insight in schizophrenia, suggesting that symptom misattribution is associated with thinness of a number of prefrontal cortical regions in both hemispheres with significant changes in the left anterior cingulate and left rostral middle frontal regions in the regression model. Our results also suggest that frontal neuroanatomical deficits are present early in the disease process, and may well be crucial to the pathogenesis of insight impairment in schizophrenia. Poor prefrontal function (in particular DLPFC dysfunction)[14] as well as poor insight at baseline[42,44] has independently been shown to predict poor outcome in FES patients. A combination of structural, functional and clinical information gathered longitudinally will be required to understand the dynamic mechanisms by which the prefrontal lobe influences outcome.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

TABLE S1. Differences in frontal cortical thickness in the regions of interest between first-episode schizophrenia patients and controls (critical threshold $P = 0.01$, after correction for multiple comparisons).

Supplementary table 1:

Frontal ROI's cortical thickness differences between FES patients and controls (critical threshold p=0.01, after correction for multiple comparisons)

Frontal region of interest	**FES (n=92)**		**HC (n=93)**		**Relative difference (%)**	**p-value**
	Mean	**SD**	**Mean**	**SD**		
Left hemisphere						
Rostral middle frontal	2.46	0.02	2.52	0.19	2.38	0.05
Caudal middle frontal	2.61	0.21	2.69	0.22	2.97	0.02
Rostral anterior cingulate	2.92	0.32	2.91	0.31	-0.34	0.71
Caudal anterior cingulate	2.71	0.25	2.74	0.27	1.09	0.40
Lateral orbitofrontal	2.60	0.25	2.65	0.23	1.88	0.15
Medial orbitofrontal	2.51	0.22	2.59	0.22	3.08	**<0.01***
Parsopercularis	2.60	0.25	2.68	0.25	2.98	0.02
Parsorbitalis	2.65	0.35	2.68	0.32	1.12	0.58
Parstriangularis	2.52	0.23	2.59	0.24	2.70	0.04
Precentral	2.39	0.22	2.43	0.21	1.64	0.20
Superior frontal	2.90	0.24	3.00	0.22	3.33	**<0.01***
Frontal pole	2.79	0.35	2.94	0.38	5.10	**<0.01***
Right hemisphere						
Rostral middle frontal	2.44	0.20	2.54	0.21	3.93	**<0.01***
Caudal middle frontal	2.60	0.23	2.67	0.24	2.62	0.04
Rostral anterior cingulate	2.91	0.03	2.89	0.03	-0.69	0.66
Caudal anterior cingulate	2.61	0.24	2.61	0.23	0.00	0.98
Lateral orbitofrontal	2.61	0.24	2.70	0.26	3.33	**0.01***
Medial orbitofrontal	2.50	0.25	2.57	0.21	2.72	.005
Parsopercularis	2.69	0.27	2.74	0.25	1.82	0.25
Parsorbitalis	2.69	0.30	2.78	0.33	3.23	0.05
Parstriangularis	2.58	0.24	2.66	0.25	3.00	0.02
Precentral	2.38	0.23	2.41	0.21	1.24	0.29
Superior frontal	2.86	0.22	2.97	0.22	3.70	**<0.01***
Frontal pole	2.76	0.28	2.88	0.42	4.17	0.04

Relative difference (RD) is the percentage difference between cortical thickness in HC and FES patients and is calculated as follows: RD = $[(mean_{HC}-mean_{FES})/mean_{HC}]$ X 100%

CHAPTER 4

CHILDHOOD TRAUMA ASSOCIATED WHITE MATTER ABNORMALITIES IN FIRST-EPISODE SCHIZOPHRENIA

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CHILDHOOD TRAUMA ASSOCIATED WHITE MATTER ABNORMALITIES IN FIRST-EPISODE SCHIZOPHRENIA

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Abstract

Schizophrenia is associated with brain connection irregularities within and between brain regions. Childhood trauma increases the risk of schizophrenia suggesting that the relationships between childhood trauma and brain connectivity requires further investigation. Here, we examine the relationship between childhood trauma (as measured by the Childhood Trauma Questionnaire) and fractional anisotropy (FA) in 53 minimally treated first-episode schizophrenia patients and 51 community matched controls. Patients who experienced high levels of trauma had significantly lower FA in the inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus (SLF), and inferior fronto-occipital fasciculus (IFOF) compared to controls who experienced high levels of childhood trauma. A history of childhood sexual abuse in patients was associated with lower FA in the IFOF, ILF, SLF and forceps major compared to patients without a history of sexual abuse. However, patients who had experienced childhood emotional neglect had higher FA in the right SLF compared to patients with low levels of emotional neglect. Our findings highlight altered cortico-limbic circuitry in first-episode schizophrenia patients compared to controls and differential effects of childhood emotional neglect and sexual abuse on white matter in patients. Although stress related WM pathways appear to be involved in both schizophrenia and otherwise healthy controls previously exposed to childhood trauma, the pattern of disruption of WM integrity in FES patients appears to be distinct.

Keywords

abuse/neglect/diffusion tensor imaging/fractional anisotropy/MRI

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1. Introduction

There is a well-established link between childhood trauma and schizophrenia. A history of childhood trauma both increases the risk of developing psychosis,[1] and is associated with greater co-morbidity,[2] more cognitive impairment[3] and persistence of symptoms over time.[4] Childhood trauma denotes a range of possible severe adverse experiences, including sexual, physical and emotional abuse, and physical and emotional neglect.[5] One possible explanation for the association is that childhood is a sensitive developmental period and childhood trauma, through psychological or biological mechanisms, interferes with normal neurodevelopment, thereby establishing a biological vulnerability in affected individuals.[6]

Indeed, adults with histories of childhood maltreatment have lower grey matter volumes in the anterior cingulate, prefrontal cortex, corpus callosum and hippocampus, higher amygdala reactivity to emotional faces and diminished striatal response to anticipated rewards than non-maltreated comparison subjects (for review see Teicher and Samson, 2016).[7] Although the biological mechanisms that underlie these associations remain unclear, the evidence to date suggests that a history of childhood maltreatment is associated with disruption of the development and functioning of the HPA axis and may sensitise neurobiological systems implicated in stress adaptation and response thereby shaping neural structure and functioning.[8–10] Childhood adversity may have a broad impact on neurodevelopment via a cascade of stress-mediated effects on hormones and neurotransmitters that shape neurogenesis, synaptic overproduction, pruning, and myelination during sensitive periods in genetically susceptible individuals, affecting stress-vulnerable brain regions such as the hippocampus, amygdala, neocortex, and white matter tracts.[7,11] Among these, white matter abnormalities in healthy participants who experienced childhood trauma complement brain morphological studies.

The microstructural properties of white matter tracts are usually studied in vivo with diffusion tensor imaging (DTI), an approach that provides a number of measures of white matter integrity, of which Fractional Anisotropy (FA) is probably the most commonly reported.[12] FA values are thought to reflect both myelination and organization of fibre tracts that form the basis

of brain connections.[13] A history of childhood trauma has been associated with white matter abnormalities in otherwise healthy participants, with a prediliction for the corpus callosum and cortico-limbic tracts.[14]

Psychiatrically healthy adults with a history of childhood trauma have shown reduced FA in the corpus callosum, corona radiata, cingulum hippocampus, inferior fronto-occipital fasciculus, superior longitudinal fasciculus and uncinate fasciculus.[14] Decreased FA in frontal and temporal white matter regions, including in the uncinate fasciculus, superior longitudinal fasciculus, and arcuate fasciculus, has been described in children with a history of early social deprivation.[15] Adolescents who had experienced early childhood neglect showed lower FA values in the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, corticospinal tract, cingulum, anterior corona radiata as well as greater FA in anterior thalamic radiation and forceps minor compared with comparison adolescents who had not experienced neglect.[16]

Considering the role of childhood trauma in increasing the risk of schizophrenia, the relationships between childhood trauma and brain connectivity in schizophrenia are of interest. Schizophrenia has been characterised as a disorder of brain connectivity,[17–20] and several studies have demonstrated a disruption in the trajectory of white matter (WM) development in psychotic and clinical high risk samples.[21–23] There is also growing evidence that childhood and adolescence are sensitive stress exposure periods when structures and pathways impacted by trauma are most vulnerable resulting in an alteration in trajectories of brain development.[7,11]

To our knowledge, there is only one known neuroimaging study that has examined the integrity of white matter tracts in people with schizophrenia who have experienced childhood trauma.[24] In a cohort of 83 patients with chronic schizophrenia, Poletti et al reported an association between an adverse early familial environment and reduced FA in the corpus callosum, left cingulum, left corona radiata, bilateral superior longitudinal fasciculus and left anterior thalamic radiation. Although these findings suggest the presence of a set of brain alterations common to both schizophrenia patients and non-clinical samples who have experienced childhood adversity, the lack of control group, the long illness duration and medication exposure limit the generalizability of these findings. Furthermore, the study used the Risky Family Questionnaire total score as a single measure of familial conflict, neglect and abuse and did not differentiate between trauma types, which may have differential effects. For example a history of childhood abuse is more pronounced for persistent positive symptoms, while neglect is associated with more general psychopathology.[25,26] There is also

some evidence that there may be a greater association between psychosis and physical abuse than with other adverse childhood experiences.[27]

This study examined whether DTI measures of WM tracts are associated with childhood trauma in first episode schizophrenia (FES). We used tract based spatial statistics (TBSS) to examine the relationship between childhood trauma and fractional anisotropy in FES patients (n=54) and healthy controls (n=51) recruited from the same geographical areas. Firstly, we hypothesized that childhood trauma related FA abnormalities would have a predilection for stress sensitive cortico-limbic tracts and the corpus callosum in patients and in controls. Next we hypothesized that these abnormalities would be greater in patients who experienced childhood trauma than in controls who experienced childhood trauma. Finally, we performed an exploratory analysis examining whether trauma subtypes had differential effects on white matter connectivity in patients.

2. Methods

2.1 Participants

The sample comprised 77 minimally treated first-episode schizophrenia patients and 51 matched controls as part of a study examining clinical, biological and functional outcome of FES in Cape Town, South Africa. Patients were recruited from inpatient services at Tygerberg and Stikland Hospital, and related community clinics in Cape Town, South Africa. For inclusion in the study, patients had to be aged 16 to 45 years, and experiencing a first psychotic episode meeting Diagnostic and Statistical Manual of Mental Diseases, Fourth Edition, Text Revisions (DSM-IV TR) diagnostic criteria for schizophrenia, schizophreniform or schizoaffective disorder based on the Structured Clinical Interview for DSM-IV (SCID) – Patient Edition.[28] The healthy control group was matched for age, sex, ethnicity and level of education (Table 1), and had no DSM-IV axis I or II disorder as determined by the by SCID-Non-Patient Edition interviews. Healthy controls were neighbourhood contacts of the families of the patients with FES and advertisements were placed in community centers in the same catchment area as the patients. Patients and controls were excluded if they had a serious or unstable general medical condition, mental retardation, current substance abuse (as confirmed by history taking of use in the past month or a positive urine drug screen), and less than 7 completed years of schooling. Patients and caregivers were interviewed to corroborate patient histories. Patients were excluded if they had a lifetime exposure to > 4 weeks of antipsychotic medication or were previously treated with a long-acting depot antipsychotic. Each patient was carefully screened with a thorough physical examination and review of the medical history, ECG, urine toxicology screen and structured assessment of symptoms to verify that inclusion criteria were met. Duration of untreated psychosis was estimated from the onset of continuous positive psychotic symptoms to initiation of adequate

treatment. Adequate treatment was defined as the start of structured treatment with antipsychotic medication. Controls were excluded if they had a first degree relative with a psychotic disorder. Patients and controls were compensated for transport costs incurred during their participation in the study, but did not receive any other financial reward.

This study was conducted according to the principles of the Declaration of Helsinki 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/). After the study procedures were fully explained in accordance with the ethical guidelines of the institutional review board, participants provided written informed consent. The parent study was registered on the South African National Clinical Trials Register (www.sanctr.gov.za/SAClinicalTrials/tabid/169/Default.aspx), trial number DOH-27-0710-1957.

2.2 Clinical rating scales

Diagnosis was assessed with the Structured Clinical Interview for DSM-IV [SCID].[28] Severity of psychotic symptoms was assessed using the complete Positive and Negative Syndrome Scale (PANSS).[29] Duration of untreated psychosis (DUP) was estimated from the onset of continuous positive psychotic symptoms to initiation of adequate treatment. Diagnosis and clinical assessment was determined by trained physicians, and inter-rater reliability testing was conducted periodically for the PANSS (intraclass correlation 0.7 or higher).

Participants and controls were assessed with the Childhood Trauma Questionnaire (CTQ) short form, a self-administered inventory that has demonstrated reliable and valid retrospective assessment of child abuse and neglect.[30] The instrument has 28 Likert-type items (25 clinical symptom items and 3 validity items to identify underreporting), and 5 subscales (sexual abuse, physical abuse, emotional abuse, physical neglect and emotional neglect). Subscale scores range from 5-25 and the scale also yields a total score which is the sum of the 5 subscales ranging from 25-125.[30] We included subscale and total scores in the analyses. Of the 77 first-episode schizophrenia patients who completed the childhood trauma questionnaire, 16 were excluded from the neuroimaging component because we applied for ethics approval for the MRI component once recruitment of patients in the parent study had already begun. A further 2 patients were lost because of scan error, 2 were excluded because of motion artefacts and 4 were unable to be scanned because of claustrophobia. Of the 52 controls, 1 was excluded due to scan error.

2.3 Image acquisition

We acquired diffusion-weighted images (DWIs) on a 3.0 T Siemens Allegra MRI scanner (Erlangen), Germany with the following parameters: field of view= 220m, spatial resolution 1.8X1.8X1.8mm^3, repetition time= 8800ms, echo time 88ms, 65 slices, no distance factor with twofold GRAPPA acceleration. The gradients were applied in 30 directions with b=1000s/mm^2 and a single unweighted volume (b=0s/mm2) were also acquired. The sequence was repeated three times.

2.4 Image pre-processing

The DWIs were pre-processed using the Functional MRI of the Brain (FMRIB) Software Library (FSL) version 4.1.8 (www.fmrib.ox.ac.uk/fsl/)[31] Raw DTI data were corrected for eddy current distortions and head motion, and the images were imported into Matlab.[32] The three acquisitions were co-registered by using the first b=0 mm/s^2 as the reference image. Outliers were determined by calculating the Z-value of the tensor estimates at the 25th and 75th percentiles. Data points falling outside of more than 3 standard deviations were discarded. The acquisitions were then averaged and exported to the FSL for further processing.

2.5 MRI analysis

Using FSL's Randomize tool, permutation-based inferences with Threshold-Free Cluster Enhancement (TFCE) were carried out for voxelwise analysis of FA data.[33] Tract-based spatial statistics (TBSS) version 1.2 was used for voxelwise analysis of the preprocessed FA data. First, individual FA images were aligned to the FMRIB58_ FA standard-space image, using nonlinear registration. Next, the mean FA image was generated and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the entire group. The mean FA skeleton was then thresholded at a FA value of ≥0.4, to exclude peripheral tracts and minimize partial voluming. Finally, each participant's aligned FA images were projected onto the mean FA skeleton and the resulting data were fed into voxelwise permutation-based analysis. The resulting statistical maps were corrected for multiple comparisons across space ($p < 0.05$) and the JHU White Matter and Juelich Histological atlases were used to label clusters with significant FA alterations.

2.6 Statistical analysis

We used analysis of t-tests and chi square tests to compare age, gender, and education in patients and controls. The CTQ total and subscales were not normally distributed; therefore, we used a median-split approach to dichotomise scores into high/low severity of trauma. For our primary analyses, we performed whole brain analyses examining for overall FA differences between all FEP patients and controls and compared FA between those who

had high levels of overall childhood trauma and those who had low levels. We thereafter performed post-hoc analyses comparing those who had high levels of trauma subtypes (sexual abuse, physical abuse, physical neglect, emotional neglect, emotional abuse) in various groups (within patients, between patients and controls and within controls). We assessed for between group (patient and control) interaction.

3. Results

3.1 Participant characteristics

The clinical and demographic features are presented in Table 1. Age, gender, education and ethnicity were similar in patients and controls. Patients and controls had comparable CTQ total and subscale scores. In keeping with the high levels of community violence experienced by our cohort, there was no significant difference in the number of patients and controls with high levels of overall trauma and trauma subtypes (reported previously).[34] FA values were significantly lower in FES patients compared to controls, as published in detail elsewhere.[35]

3.2 Fractional anisotropy and childhood trauma in patients and controls

3.2.1 Patients versus controls

Total childhood trauma: Compared to matched controls who experienced high levels of CT, FES patients who experienced high levels of CT had significantly lower FA in a cluster centred in the left inferior longitudinal fasciculus (ILF), and superior longitudinal fasciculus (SLF), as well as a cluster in the right SLF and inferior fronto-occipital fasciculus (IFOF) (Table 2).

Childhood trauma subtypes: There was no difference in FA between patients and controls who experienced high levels of childhood trauma for any of the subscales.

3.2.2 Within patient group

Total childhood trauma: There was no significant difference in FA in any tract between FES patients who experienced high levels of overall CT and FES patients who experienced low levels of CT.

Childhood trauma subtypes: FES patients with high levels of childhood sexual abuse had *lower* FA in a cluster comprising the right IFOF, ILF, and forceps major compared to FES patients without a history of sexual abuse (Table 3). On the other hand, FES patients with a history of high levels of childhood emotional neglect, had *higher* FA in the right SLF compared to FES patients with low levels of childhood emotional neglect (Table 4). There were no significant differences in FA between FES patients who experienced high levels of physical neglect, physical abuse and emotional abuse compared to FES patients who experienced low levels.

3.2.3 Within control group

Total childhood trauma: There was no significant difference in FA in any tract between controls who experienced high levels of overall CT and controls who experienced low levels of CT.
Childhood trauma subtypes: There was no difference in FA between controls who experienced high levels and those controls who experienced low levels of childhood trauma in any of the subscales.

4. Discussion

Here, we investigated associations between white matter FA and childhood trauma in people with FES and in matched community controls. A key finding of our study is that FA of the ILF, SLF and IFOF is lower in childhood trauma exposed patients than in childhood trauma exposed controls. These WM tracts have been shown to be compromised in schizophrenia[36,37] and also known to be affected by childhood trauma[7,14,38,39] which may point to biological markers of vulnerability and resilience to schizophrenia in childhood trauma exposed individuals.

Our findings are in keeping with the only known previous study that examined DTI abnormalities related to childhood adversity in schizophrenia.[24] The authors found that, in 83 patients with chronic schizophrenia, higher exposure to harsh parenting was associated with lower FA in the bilateral SLF, left ILF, left cingulum, corpus callosum, left cingulum, left corona radiata, and left anterior thalamic radiation, although the lack of control group and illness chronicity limits the generalizability of these findings.

The disconnection hypothesis proposes that, in schizophrenia, there is dysfunctional integration in distributed but circumscribed neuronal systems that leads to neuromodulatory failure, e.g. mesocorticolimbic dysconnectivity.[40] The ILF, SLF and IFOF are important cortico-limbic tracts that are implicated in the pathophysiology of schizophrenia.[41,42] The IFOF directly interconnects the occipital, posterior temporal and the orbito-frontal areas while the ILF connects similar brain areas indirectly, and is an important source of fibres afferent to the amygdala and hippocampus.[43–45] The amygdala, hippocampus, prefrontal cortex and related pathways are integral to emotional response, fear modulation and memory. The involvement of the ILF, SLF and IFO in both childhood trauma and schizophrenia suggests that limbic circuitry may be particularly vulnerable to long-term consequences of childhood maltreatment in schizophrenia.

A further important finding of our study is that FA was lower in FES patients with a history of childhood sexual abuse compared to FES patients without a history of sexual abuse.

Furthermore, FES patients who had experienced childhood emotional neglect had higher FA compared to patients without emotional neglect. These findings are consistent with the proposal that trauma types have differential effects on neurodevelopment and that childhood abuse and neglect lie along independent pathways to psychosis.[25,26] McLaughlin and colleagues[46] suggest that threat (e.g. sexual abuse) and deprivation (e.g. emotional neglect) are distinct dimensions of the environmental experience and may have distinct effects on neural development.

FES patients with a history of sexual abuse had lower FA in clusters involving the IFOF, ILF, SLF and forceps major than patients without a history of sexual abuse. Whether sexual abuse exerts effects that can be differentiated from the effects of physical and emotional abuse remains to be determined. It could be speculated that sexual abuse is particularly pernicious and impactful on the developmental trajectory, resulting in specific neuroplastic adaptive changes. These adaptations, for example reduced synaptic density, may initially be protective in that it shields a child by gating sensory processing.[47] However, later in life, these impaired neurobiological substrates may predispose to the development of disorders.[47]

The finding of higher FA in the SLF in FES patients who experienced childhood emotional neglect compared to patients who did not experience emotional neglect is unanticipated, but does lend support to the theory that childhood abuse and neglect may lie on independent neurobiological pathways to psychosis. [25,34,48]

The superior longitudinal fasciculus (SLF) is the largest association tract, connecting the frontal lobe cortex to parietal, temporal and occipital lobe cortices.[49] The seemingly counterintuitive finding of increased FA may in part be explained by the neurodevelopmental model of schizophrenia and the role of aberrant strengthening and pruning of synaptic connections associated with schizophrenia. Exposure to early stress may prompt adaptive brain development along alternative developmental pathways to enable survival in a stress filled world.[11] This may be especially relevant to the experience of emotional neglect which is characteristically chronic in that there is a long-standing lack of parent-child attachment.[50] It may be that a child develops adaptive responses to emotional neglect such as heightened sensitivity to interpersonal stress and avoidant coping mechanisms which may later be relevant factors implicated in the development of schizophrenia. Excessive pruning shown to occur in schizophrenia may lead to the preservation of exuberant connections as a result of childhood neglect which may thereafter contribute to symptom generating neural activity in people who develop schizophrenia. SLF is a late maturing tract and so may be differentially vulnerable to the effects of emotional neglect.

Strengths of our study include the inclusion of first-episode, minimally treated patients, which allowed us to largely eliminate the effect of treatment and illness chronicity as well as the inclusion of matched community controls who reported similar levels of childhood trauma exposure. We assessed the effect of different types of childhood trauma and used a well-validated measurement of childhood trauma. Several limitations should be noted. The CTQ is a retrospective self-rated instrument and nature of the trauma could not be explored in depth, nor does it assess the frequency of trauma and the age at which trauma first occurred. Further the CTQ does not assess other forms of childhood adversities such as witnessing domestic violence and bullying, the latter is hypothesized to be associated with psychosis.[51] We did not assess for the interaction effects of gender, ethnicity and substance use on whole brain analyses. Although we did not find a relationship between these variables and childhood trauma in patients and controls, these factors have been shown to be related to childhood trauma in other studies.[50]

This study provides important preliminary data that increase our understanding of the relationship between childhood trauma and schizophrenia. Our findings highlight altered cortico-limbic circuitry in FES patients compared to community controls and differential effects of childhood emotional neglect and sexual abuse on white matter in FES patients. Although previous studies have found that stress related WM pathways appear to be involved in both chronic schizophrenia[24] and otherwise healthy controls previously exposed to childhood trauma,[14–16] the pattern of disruption of WM integrity in FES patients appears to be distinct.

Future studies should focus on resilience factors to address why some individuals exposed to childhood trauma develop schizophrenia while others do not. It is also important to explore whether brain changes associated with childhood trauma in schizophrenia patients are correlated with clinical, cognitive and functional outcomes, while taking into account the possible confounding effects of age of onset of childhood trauma, cumulative trauma load, gender, age of onset and duration of psychosis, and treatment status. It would also be important to examine whether clinical symptoms in patient vary according to trauma subtypes and whether these differences influence DTI measurement. Finally, future research should consider critical periods during which the brain is particularly susceptible to both the impact of childhood trauma as well the development of schizophrenia.

Table 1: Demographic and clinical characteristics for first-episode schizophrenia and control groups

Characteristic	**All participants**		**Analysis**		
	FES (n=54)	**Controls (n=51)**			
			test statistic	df	p
Male sex, n (%)	40(74)	35(67)			.07
Age (mean, SD)	24.78 (6.98)	25.04 (6.85)	t=.96	103	.35
Education (years)	9.9 (1.9)	10.3 (1.5)	t=-1.93	103	.11
Ethnicity n(%)			x^2=2.99		.22
Black	0 (0)	6(15.79)			
Mixed race	15 (93.75)	29 (76.31)			
White	1 (6.25)	3 (7.89)			
CTQ, median [range]					
Emotional neglect	12[5-25]	9.5[5-24]	z=-.821		.41
Physical abuse	7[5-23]	7[5-25]	z=-.194		.84
Emotional abuse	9[5-22]	9[5-25]	z=.413		.68
Physical neglect	9[5-22]	8.5[5-17]	z=-.88		.38
Sexual abuse	5[5-25]	5[5-25]	z=.390		.70
Total score	46.5[25-92]	43[25-93]	z=-.225		.82
CTQ, High (%)*					
Emotional neglect	17(32.69)	14.26.92)			.64
Physical abuse	20(38.46)	15(28.8)			.41
Emotional abuse	15(28)	13(25.0)			.96
Physical neglect	26(48.15)	22(42.31)			.65
Sexual abuse	13(24.53)	14(26.92)			.85
Total score	16(29.63)	13(25.00)			.70
	FES only (n=54)				
	CTQ total high (n=16)	CTQ total low (n=38)			
Age (mean, SD)	25.54 (7.68)	22.56 (5.42)	t=1.68	52	.10
Male sex, n(%)	13 (81.25)	27 (71.05)	x^2=0.61		.43
Ethnicity n(%)			x^2=2.99		.22
Black	0 (0)	6(15.79)			
Mixed race	15 (93.75)	29 (76.31)			
White	1 (6.25)	3 (7.89)			
Education (years)				52	
Substance abuse	9(56.25)	15(39.47)	x^2=1.28		.26
PANSS total score	94.2 (11.71)	92.32(14.53)	t=-1.16	51	.28
Antipsychotic naïve	8 (53.33)	18 (47.37)	x^2=.15		.70
DUP	39.39(46.80)	29.15(32.35)	t=-.91	51	.37

Table 2: Whole brain *lower* fractional anisotropy in FES patients with history of childhood trauma compared to FES matched controls with a history of childhood trauma.

Brain region	Tracts	Hem	Cluster size	MNI coordinates of voxel of maximum significance[a]			Probability
				x	*y*	*z*	
Cluster 1	Inferior longitudinal fasciculus, Sup long fasciculus (temporal part)	L	34	125	77	99	.04
Cluster 2	Sup long fasciculus (temporal part), Inferior fronto-occipital fasciculus	R	214	59	87	86	.04

Table 3: Whole brain *lower* fractional anisotropy in FES patients with history of childhood sexual abuse compared to FES patients without a history of sexual abuse

Brain region	Tracts	Hem	Cluster size	MNI coordinates of voxel of maximum significance[a]			Probability
				x	*y*	*z*	
Cluster 1	Inferior fronto-occipital, inferior longitudinal fasciculus, forceps major	R	971	61	58	85	.006
Cluster 2	Forceps major, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, superior longitudinal fasciculus	L	2793	111	74	84	.007

Table 4: Whole brain *higher* fractional anisotropy in FES patients with history of childhood emotional neglect compared to FES patients without a history of emotional neglect

Brain region	Tracts	Hem	Cluster size	MNI coordinates of voxel of maximum significance[a]			Probability
				x	*y*	*z*	
Cluster 1	Superior longitudinal fasciculus	R	4	63	84	115	.0001
Cluster 2	Superior longitudinal fasciculus	R	10	64	80	112	.0001
Cluster 3	Superior longitudinal fasciculus	R	21	53	93	100	.0001
Cluster 4	Superior longitudinal fasciculus, Sup longitudinal fasc (temporal part)	R	165	59	86	107	.001

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CHAPTER 5
ASSOCIATIONS BETWEEN GLOBAL BRAIN MEASURES AND STATE- AND TRAIT-RELATED SYMPTOM EXPRESSION OF SCHIZOPHRENIA

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ASSOCIATIONS BETWEEN GLOBAL BRAIN MEASURES AND STATE- AND TRAIT-RELATED SYMPTOM EXPRESSION OF SCHIZOPHRENIA

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Abstract

The course of schizophrenia is characterised by episodes of psychotic symptoms (which may be largely state related) and enduring deficits of negative symptoms, cognition and functioning (which may be largely trait related). Neuroimaging holds the promise that brain deficits may serve as a biomarker for illness detection and treatment outcome. We investigated the relationship between global brain measures and trait-related symptoms (endpoint scores), and global brain measures and state-related symptoms (change scores). We examined global cortical, subcortical and white matter volume, and global cortical thickness in 54 first-episode minimally treated schizophrenia patients at baseline. We performed clinical, cognitive, and neurological assessments at baseline and twelve-month follow-up. Baseline subcortical gray matter volume was significantly associated with sensory integration (0.02) and verbal learning (0.04) trait scores, cortical volume with verbal learning (0.04) trait scores, cortical thickness with social and occupational functioning (0.03) trait scores, and white matter volume with motor coordination (0.007) state scores. The differential associations between state and trait related symptoms and brain structure indicate that future studies assessing the neurobiological underpinnings of the illness should take care to differentiate between state and trait related manifestations.

Keywords

Cortical thickness/white matter volume/gray matter volume/subcortical volume/MRI

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1. Introduction

The course of schizophrenia is typically characterised by episodes of psychosis superimposed on enduring deficits including negative symptoms and cognitive and psychosocial functional impairments (Andreasen et al. 2005). The degree to which symptoms can be categorised as being state or trait related, or mixed, has been a focus of attention as it would allow a better understanding of the neurobiology of the illness and the possibility to improve our ability to detect, treat and ultimately prevent the disorder. Typically, psychotic symptoms (delusions and hallucinations) may be largely state-related, whereas negative and cognitive symptoms may be largely trait-related. However, the distinction is not clear-cut as most-symptoms display both state-and trait-related features insofar as they improve with treatment, but persist to varying degrees as well. State markers reflect clinical manifestations of psychotic exacerbations whereas trait markers are typically enduring and relatively independent of state-related fluctuations in the individuals' status. Trait markers could be brought about primarily by the expression of genes and are considered to be linked to the core abnormalities that play an antecedent and possibly causal role in the illness (Braff et al. 2006).

Imaging studies have consistently demonstrated differences in brain morphology in people with schizophrenia compared with healthy volunteers. Brain structural alterations are present early in the illness and may predate symptom onset (Mechelli et al. 2011; Dazzan et al. 2011). Abnormalities seen in first-episode schizophrenia patients when compared to controls are diffuse, comprising reductions of cortical and subcortical gray matter and white matter volumes (Haijma et al. 2012), as well as differences in cortical thickness (thinning and thickening) (Xiao et al. 2013). These neuroimaging findings offer some hope that brain deficits may serve as potential biomarkers for illness detection and treatment outcome.

Efforts to understand the neurobiological basis of the symptom expression of schizophrenia by examining associations between symptoms and brain structure, have been largely unsuccessful (Mathalon and Ford 2012). Although there is some indication from a recent systematic review that medial temporal and prefrontal cortical areas and related connections to subcortical regions may be related to outcome (Dazzan et al. 2015), there is considerable variability in findings. These inconsistencies are likely, at least in part, due to methodological shortcomings. For example, studies were frequently conducted in small patient samples, in different phases of illness (first-episode and multi-episode), and using cross-sectional designs. Also, and of particular importance when considering state-and trait related manifestations of the illness, the clinical status of patients and duration of exposure to treatment has varied (Mathalon and Ford 2012). To accurately assess state-related features of illness, initial assessments need to be conducted in the acute, unmedicated state; and for

assessment of trait-related features, patients should have received the benefit of effective treatment for a sufficient period of time before symptoms are regarded as persistent.
In this study, we carefully characterised the state and trait related components of the symptom expression of first-episode schizophrenia and determined their relationships with global cortical thickness and global brain volume (cortical volume, subcortical volume, white matter) measures. We defined symptom expression broadly to include measures of psychopathology, insight, functionality, neurological signs and cognition. We conducted baseline assessments in the acute, largely unmedicated state and then again following treatment with depot flupenthixol medication according to a standard protocol for 12 months. We hypothesised that global brain volume and cortical thickness measures would show stronger associations with trait-related (endpoint scores) rather than state-related symptoms (change from baseline to endpoint scores). Specifically, we hypothesised that endpoint negative, cognitive and neurological symptoms would have the more significant correlations with global brain volume and cortical thickness measures.

2. Methods

2.1 Overview

This was a substudy of a longitudinal study assessing outcome in patients with a first-episode of schizophrenia or related disorder who were treated for 12 months with the lowest effective dose of flupenthixol decanoate according to a standard protocol. We obtained ethics approval from the Human Research Ethics Committee of Stellenbosch University. The study was conducted according to the International Conference on Harmonization good clinical practice guidelines (Dixon 1999). The parent study was registered on the South African National Clinical Trials Register, trial number DOH-27-0710-1957, (www.sanctr.gov.za/SAClinicalTrials/tabid/169/Default.aspx). By selecting first-episode, minimally treated patients we minimised effects of illness chronicity and previous treatment. We conducted baseline assessments in the acute, largely unmedicated state, enabling us to capture the full range of symptoms prior to treatment effects. We treated patients according to a standard protocol with depot flupenthixol decanoate, thereby removing possible confounding differential effects of antipsychotics and covert non-adherence. We excluded patients who received treatment for less than 6 months, to ensure that endpoint assessments reflected an adequate treatment period for acute symptoms. We considered baseline assessments to represent a combination of state and trait related features.

2.2 Participants

Patients were recruited from psychiatric hospitals and clinics in Cape Town and surrounds between April 2007 and March 2011. They were all voluntary patients, and provided written,

informed consent to participate in the study. In the case of minors, we obtained consent from the legal guardian. Inclusion criteria for the parent study were men and women, aged 16–45 years, experiencing a first psychotic episode meeting Diagnostic and Statistical Manual of Mental Diseases, Fourth Edition, Text Revisions (DSM-IV TR) (First 1994) diagnostic criteria for schizophrenia or schizophreniform disorder. Exclusion criteria were lifetime exposure to > 4 weeks of antipsychotic medication, treatment with a long-acting depot antipsychotic, serious or unstable general medical condition, current substance abuse, an educational level <Grade 7 and contraindications for MRI scanning. For the present study, patients were included if they had a baseline structural MRI scan as well as clinical and cognitive data obtained at baseline and after at least six months of treatment. All patients were scanned within one week of recruitment into the study.

2.3 Treatment

Patients received oral flupenthixol 1–3 mg/day for 7 days, followed by flupenthixol decanoate injections 2-weekly for the study duration. Initiation dose was 10 mg 2-weekly, with 6-weekly increments of 10 mg 2-weekly IMI permitted, to a maximum of 30 mg 2-weekly IMI. Additional oral flupenthixol was permitted at the discretion of the investigator, as was lorazepam, anticholinergics, propranolol, antidepressants and medication for general medical conditions. No benzodiazepines, propranolol or anticholinergics were permitted in the 12 h prior to assessments.

2.4 Clinical Evaluations

Symptom expression was broadly defined and included measures of psychopathology (positive, negative, disorganised and excitement/hostility domains, depression), insight, functionality, neurological signs and cognition. Patients were assessed with the Structured Clinical Interview for DSM-IV (SCID) (First et al. 2012). Severity of psychopathology was assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987). We used factor-analysis derived symptom domains for positive, negative, depression/anxiety, excitement/hostility and disorganised symptoms (Emsley et al. 2003). Depressive symptoms were assessed with the Calgary Depression Rating Scale for Schizophrenia (CDSS) (Addington et al. 1994). We administered the Clinical Global Impressions (CGI) Scale as an overall clinician determined summary measure (Guy 1976). Neurological soft signs were assessed with the Neurological Evaluation Scale (NES) (Buchanan and Heinrichs 1989). The NES is a structured clinical battery consisting of 26 items and provides three subscale scores reflecting dysfunction in sensory integration, motor coordination and motor sequencing as well as a NES total score. Insight was measured by means of the Birchwood Insight Scale (BIS) (Birchwood et al. 1994) which assesses three dimensions of insight: symptom awareness, illness awareness, and need for treatment, as well as providing a BIS total score. Functionality was assessed with the Social and Occupational Functioning Assessment Scale

(SOFAS) (Morosini et al. 2000). Investigators underwent training and inter-rater reliability (IRR) testing. The IRR was >0.75 for all scales.

2.5 Cognitive evaluations

Cognitive performance was assessed by the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) Cognitive Consensus Battery (MCCB), developed to measure cognitive functioning in schizophrenia. The MCCB measures seven cognitive domains (speed of processing; attention/vigilance; working memory; verbal learning; visual learning; reasoning and problem solving; and social cognition) and a composite score (Nuechterlein et al. 2008). The MCCB was administered by trained psychologists.

2.6 State vs. trait assessments

We assumed that clinical and cognitive scores in the acute state represent both state and trait components, while those in clinically stable patients represent trait components, and the degree of change from the acute state to stable state would represent state components. Therefore, for state-related clinical and cognitive variables we used change scores (endpoint-baseline score). For trait-related clinical and cognitive variables we used endpoint scores, calculated by last observation carried forward.

2.7 Imaging methods

Patients underwent baseline scans before receiving any flupenthixol medication. We acquired high-resolution T1-weighted data on a 3T Siemens Allegra MRI scanner (Erlangen, Germany) with the following acquisition parameters: MPRAGE sequence, 2080 ms repetition time; 4.88 ms echo time, Field of view: 230 mm, 176 slices, 0.9 mm X 0.9 mm X 1 mm voxel size. Scans were screened for intracranial pathology and inspected for motion artefacts. Scans were processed and analyzed using Freesurfer stable release version 5.1. (http://surfer.nmr.mgh.harvard.edu/). Slices were resampled to a three-dimensional image with 1mm isotropic voxels. Non-uniform intensity normalization was performed and images were registered to the Montreal Neurological Institute (MNI) space. A second normalization step was performed with a different algorithm in which control points were automatically identified and normalized to a standard intensity value. Following an automated skull strip procedure, gross brain anatomy was delineated into cortical and subcortical labels. Reconstructions were performed with custom batching scripts, on the Centre for High Performance Computing (CHPC) Rosebank, Cape Town, Sun Intel Nehalem cluster (http://www.chpc.ac.za/). Skull stripping, Talairach transforms, atlas registration, spherical surface maps and parcellations were initialized with common information from the within-subject template. All data were visually inspected for errors in Talairach transformation, skull

strip, final segmentations as well as the within subject-registrations. Any errors were corrected manually and re-inspected.

2.8 Global brain measures

We selected the following global measures of brain volume provided with Freesurfer (http://surfer.nmr.mgh.harvard.edu/): (1) Cortical volume – a surface-based volume calculation, comprising the volume inside the pial surface minus the volume inside the white surface minus tissue inside the ribbon that is not part of cortex. (2) Subcortical gray matter volume – a voxel count of the thalamus, caudate, hippocampus, amygdala, accumbens, ventral diencephalon and substantia nigra. (3) White matter volume – using surface-based volume computation for part of the calculation and voxel counts to subtract anything that is not white matter from the volume inside the white surface. We calculated global cortical thickness using a weighted composite measure of Freesurfer defined cortical thickness regions of interest.

2.9 Statistical analyses

We assessed the distribution of the data by inspection of histograms and normal probability plots. We conducted Pearson correlational coefficient analyses to investigate both state (change scores) and trait-related (endpoint scores) associations between baseline cortical thickness, cortical volume, white matter volume and subcortical gray matter volume and clinical and cognitive variables change and endpoint scores. Thereafter, we used hierarchical multiple regression to predict brain measures (cortical gray volume, subcortical gray volume, white matter volume, cortical thickness). For each of the brain measures, we ran separate models for change-related and state-related variables. First, an intracranial volume block was entered to allow the dependent brain volume variable to be adjusted for intracranial volume (this was not performed for cortical thickness). Second, a demographic block comprising patient age, education, and sex was entered. Third, a clinical block comprising scores on clinical assessments that correlated significantly with brain measures (correlation analyses in Supplementary table 1) was entered. Finally, a cognitive block comprising MCCB scores that significantly correlated with brain measures (correlation analyses in Supplementary table 2) was entered. The significance of each successive block's contribution to validity was assessed with F tests. All tests were 2-tailed.

3. Results

3.1 Participant characteristics

Demographic details, baseline and endpoint scores for the clinical and cognitive measures and brain volumetric measures for the 54 participants are provided in Table 1 and Table 2. Over the course of 12 months, PANSS scores (total and subscale), CDSS, GAF, CGI, SOFAS and MCCB cognitive scores improved (table 2). No significant improvement was

noted in neurological symptoms (as assessed by the NES), and insight (as measured by the BIS).

3.2 Correlations

Results for the correlation analyses between brain volume and cortical thickness measures and state-related (change) and trait-related (endpoint) scores are provided in Supplementary Table 1 and 2 respectively. Clinical and cognitive variables that correlated with brain volume and cortical thickness measures at uncorrected p<0.1 qualified for entry into the hierarchical regression models, summarised as follows:

State-related (change) scores: White matter volume: processing speed (r^2.35, p=.01), visual learning (r^2 .26, p=.07), social cognition (r^2=.29, p=.04), total insight (r^2=-.26, p=.06). Cortical volume: verbal learning (r^2=.27, p=.06), working memory (r^2 .44, p=.001), visual learning (r^2 =.23, p=.09), social cognition (r^2=.34, p=.01). Subcortical volume: processing speed (r^2=.37, p=.007), social cognition (r^2=.35, p=.01), total insight (r^2=-.33, p=.02). Cortical thickness: nil.

Trait-related (endpoint) score: Cortical volume: sensory integration (r^2=-.32, p=.02), working memory (r^2=.31, p=.02), verbal learning (r^2=.31, p=.02), illness awareness (r^2=-.27, p=.05) White matter volume: sensory integration (r^2=-.34, p=.01), processing speed (r^2=.30, p=.03), working memory (r^2=.38, p=.005), verbal learning (r^2=.31, p=.02), visual learning (r^2=.27, p=.05), reasoning and processing (r^2=.24, p=.09), social cognition (r^2=.37, p=.007), illness awareness (r^2=-.29, p=.03). Subcortical volume: sensory integration (r^2=-.24, p=.09), processing speed (r^2=.28, p=.04), working memory (r^2=.27, p=.05), verbal learning (r^2=.32, p=.02), social cognition (r^2=.40, p=.003), subcortical gray (r^2=-.14, p=.31). Cortical thickness: social and occupational functioning scale (r^2=0.27, p=.048).

3.3 Regression models

The results of the hierarchical regression analyses for predictors of cortical, white matter and subcortical gray matter volume are presented in table 3. As expected, intracranial volume and demographic variables accounted for a significant portion of variance in in cortical thickness and in cortical, subcortical and white matter volume.

3.3.1 Clinical predictors of brain volume

The block of state-related (change score) clinical predictors accounted for 8% of variance in white matter volume, while the block of trait-related (endpoint score) clinical predictors accounted for 7% of variance in subcortical volume. Within the clinical block of predictors, state-related (change) motor coordination scores as measured by the NES contributed significantly to the prediction of white matter volume (b = -.02, t = -2.80, P=0.007) and trait-related (endpoint) sensory integration scores as measured by the NES contributed significantly to the prediction of subcortical gray matter volume (b = -.017, t = -2.32, P=0.02).

Trait social and occupational functioning significantly predicted cortical thickness (b= .003, t = 2.21, P=0.03).

3.3.2 Cognitive predictors of brain volume

The block of trait-related (endpoint) cognitive scores accounted for 15% of variance in subcortical volume and 13% of variance in cortical volume. Within the block of cognitive predictors, trait-related verbal learning scores significantly predicted both cortical volume (b = .003, t = 2.04, P=0.04) and subcortical volume (b = .003, t = 2.01, P=0.04).

4. Discussion

In this study, in which the state and trait related components of the symptom expression of schizophrenia were carefully characterised, we found limited but differential associations between state and trait related symptoms and global brain measures on predictive modelling. Baseline subcortical gray matter volume was significantly associated with sensory integration and verbal learning trait scores, cortical volume with verbal learning trait scores, cortical thickness with social and occupational functioning trait scores, and white matter volume with motor coordination state scores.

In our study, the trait-related cognitive domain of verbal learning was a significant predictor of both cortical and subcortical volume. Verbal learning and memory are considered the most prominent cognitive impairments observed in schizophrenia (Manglam and Das 2013) and have been associated with reduced gray matter volume in the cingulate cortex, juxtapositional lobule, right superior temporal gyrus, and precuneus (Rannikko et al. 2012). Our findings suggest that impaired verbal learning may be the cognitive domain that is particularly trait-related, and therefore presumably closest to the neurodevelopmental deficit underlying schizophrenia.

We found that the trait-related component of sensory integration predicted cortical and subcortical gray matter volumes while the change-related component of motor coordination predicted white matter volume in the hierarchical regression analyses. Neurological soft signs are not static but vary over the course of the disorder. They improve with antipsychotic treatment although not to the level observed in healthy controls. As such, they are regarded as having both state and trait characteristics, and are proposed to be useful in identifying subjects at risk to develop schizophrenia and to monitor disease progression (Bachmann et al. 2014). Neurological soft signs have also been related to structural brain deficits in schizophrenia. A meta-analysis of structural MRI studies reported their association with smaller precentral gyrus, cerebellum, inferior frontal gyrus and the thalamus (Zhao et al. 2013). Our results suggest that the state and trait components of neurological soft signs may be differentially related to brain structure. Future studies could investigate the value of motor-

coordination change scores in monitoring disease progression, as well as a region of interest approach to address the possibility that some symptoms are related to circumscribed rather than general brain structural deficits.

We also found that social and occupational functioning trait scores significantly predicted global cortical thickness. Other first-episode schizophrenia studies have found that smaller baseline gray matter volume in the left orbitofrontal and frontopolar cortex (Kasparek et al. 2009) as well as baseline volume of the left dorsolateral prefrontal cortex (Prasad et al. 2005) predicted poorer functioning at one year follow-up. These studies are limited by small sample sizes, and to our knowledge there are no other studies that have explored cortical thickness and functioning in schizophrenia. Specific cognitive and emotional functions regulated by the prefrontal lobe may be critical in outcome (Dazzan et al. 2015). Future studies should consider a region of interest analysis investigating the relationship between cortical thickness and functioning taking into account other potential mediators, such as depression and cognition.

While several other significant associations were identified in the bivariate correlational analyses, most of these associations were lost when we controlled for age, gender, educational status and intracranial volume. Most notably, none of the measures of psychopathology or insight were significantly associated with global brain volume or cortical thickness measures. Limited significant associations with symptom expression is not entirely unexpected, as studies to date investigating structural brain measures and symptom dimensions have reported mixed findings. Thus, positive symptoms have been associated with reductions in brain morphology in some studies (Barta et al. 1990; Flaum et al. 1995; Nestor et al. 2007; Lui et al. 2009; Nesvåg et al. 2009; Padmanabhan et al. 2014; Zhang et al. 2014; Walton et al. 2017a) but not in others (Turetsky et al. 1995; Fannon et al. 2000; Molina et al. 2003). Similarly, negative symptoms have been associated with brain morphology reductions in some studies (Nestor et al. 2007; Padmanabhan et al. 2014; Zhang et al. 2014;Turetsky et al. 1995; Molina et al. 2003; Baaré et al. 1999;Benoit et al. 2012; Nenadic et al. 2015;Walton et al. 2017b) but not others (Barta et al. 1990; Flaum et al. 1995;Lui et al. 2009; Fannon et al. 2000), and some studies even found less reduction in brain structures in patients with prominent negative symptoms compared to those without negative symptoms (Nesvåg et al. 2009; Lacerda et al. 2007; Volpe et al. 2012). Finally, disorganisation symptoms have been associated with brain morphological reductions in two studies (Lui et al. 2009; Molina et al. 2003), while two others found no association (Flaum et al. 1995; Zhang et al. 2014).

There are several possible explanations for these inconsistent findings. First, the phase of illness and extent of exposure to treatment may influence results, as brain volume, cortical thickness and symptoms change over time. Progressive brain volume reductions (Ho et al. 2011) and cortical thinning (van Haren et al. 2008) have been reported, particularly in the early years of illness. These reductions have been associated with both illness progression (Ziermans et al. 2010) and medication exposure (Fusar-Poli et al. 2013). Indeed, it was for these reasons that we obtained the MRI scans at baseline, before initiation of study treatment. It is therefore possible that associations between symptom expression and brain volume and cortical thickness may become more apparent as the illness progresses. In this regard, it has been proposed that there may be processes such as inflammation that, in the early stages of illness, lead to structural enlargement through water retention (Padmanabhan et al. 2014). However, counting against this is that findings in other first-episode samples are also inconsistent. Of five such studies, one study reported associations between brain structure reductions and positive symptoms only (Lui et al. 2009), another with negative symptoms and disorganised symptoms (Molina et al. 2003), another with negative symptoms only (Benoit et al. 2012), another found no associations (Fannon et al. 2000), and one study reported increased brain volumes associated with negative symptoms (Lacerda et al. 2007).

Second, various methodological differences could explain the inconsistencies in findings to date. For example, most studies investigated specific rather than global brain regions, and these regions varied from study to study. Different measures of brain structure (e.g. measures of cortical and subcortical volumes, cortical thickness, ventricular volumes) were also employed. In addition, different instruments were used to assess symptoms and patients were at various stages of treatment response. Finally, and most likely, our findings, together with those of most previous studies, suggest that brain morphology and symptom expression in schizophrenia are only weakly associated (Crespo-Facorro et al. 2009; Crespo-Facorro et al. 2007), and the neurobiological underpinnings of the clinical manifestations of illness may not be adequately captured by structural measures (Padmanabhan et al. 2014).

Our failure to find an association between insight and brain measures could be explained by insight being related to regional rather than global brain structural deficits. Indeed, in a previous study of ours in a cohort overlapping that of the present study we found that symptom misattribution was associated with reduced left rostral middle frontal thickness and left anterior cingulate thickness specifically (Asmal et al. 2016). A systematic review of neurobiological correlates of insight impairment in schizophrenia identified the prefrontal cortex, cingulate cortex, regions of the temporal and parietal lobe and hippocampus as possible neural correlates of insight (Xavier and Vorderstrasse 2016).

Limitations of our study include the relatively small sample and the limited study duration. Assessing changes in symptom expression and brain volumes over a longer period than 12 months may yield different results. In addition, using endpoint scores as a measure of trait-related features of the illness may not be entirely accurate, as factors such as medication side-effects and substance abuse may contribute to persistent symptoms.

In conclusion, although significant associations between brain structure and symptom expression were few, the differential associations between state and trait related symptoms and brain structure indicate that future studies assessing the neurobiological underpinnings of the illness should take care to differentiate between state and trait related manifestations.

Tables

Table 1: Demographic and brain volume characteristics at baseline in FES patients (n=54)

Variable	
Age in years (mean, SD)	24(5.8)
Ethnic group (N, %)	
mixed	45 (83)
black	5 (9)
white	4 (8)
Male: female	2
Highest school grade (mean, SD)	10 (2)
DSM diagnosis (N, %)	
Schizophrenia	36
Schizophreniform	18
DUP days (mean, SD)	206 (222.2)
Endpoint flupenthixol dose (mean, SD)	13(6.3)
Global brain measures	
Cortical volume, in mm^3 (mean, SD)	444486 (52882.2)
White matter volume, in mm^3 (mean, SD)	489154 (60011.0)
Subcortical gray matter volume, in mm^3 (mean, SD)	187672 (15087)
Intracranial volume, in mm^3 (mean, SD)	1394632 (182322.9)
Cortical thickness, in mm^2 (mean, SD)	2.44 (0.13)

Table 2: Baseline and endpoint clinical and cognitive scores in FES patients (n=54)

Variable	Baseline score		Endpoint score		T	p-value
	Mean	SD	Mean	SD		
NES						
Total	13	6.7	8	5.4	6.09	<0.001
Sensory integration	2	2.2	1	1.8	3.80	<0.001
Motor coordination	1	1.3	1	1.1	3.50	<0.001
Motor sequencing	3	2.3	1	1.8	4.01	<0.001
PANSS						
Total	93	15.9	47	12.9	20.89	<0.001
Positive	13	2.9	4	2.4	16.48	<0.001
Negative	17	4.6	10	4.0	11.6	<0.001
Excitement/hostility	8	3.9	5	1.4	6.79	<0.001
Disorganised	18	4.2	9	3.4	18.77	<0.001
Depression/anxiety	9	4.3	6	2.6	6.73	<0.001
CDSS total	4	4.3	2	3.1	3.59	<0.001
SOFAS	45	12.7	65	12.4	-12.18	<0.001
BIS						
Total	8	5.4	6	2.1	-0.10	0.54
Symptom attribution	2	1.0	2	1.1	0.30	0.38
Illness awareness	2	1.3	2	1.3	0.27	0.39
Need for treatment	2	1.0	2	1.0	-0.87	0.81
MCCB						
Processing speed	22	16.3	30	16.3	-4.77	<0.001
Attention and vigilance	28	11.9	38	9.3	-5.50	<0.001
Working memory	27	16.2	36	12.8		
Reasoning and problem solving	33	9.6	40	11	-4.82	<0.001
Verbal learning	35	9.3	39	8.6	-2.80	0.003
Visual learning	33	15.9	40	13.6	-3.47	<0.001
Social cognition	28	12.8	40	11.0	-1.74	0.04
Composite score	17	15.4	27	15.1	-5.86	<0.001

NES, Neurological Evaluation Scale; PANSS, Positive and Negative Syndrome Scale; CDSS,
Calgary Depression Scale for Schizophrenia; SOFAS, Social and Occupational Functioning Assessment Scale; MCCB, MATRICS Consensus Cognitive Battery.

Table 3: Hierarchical regression analysis for predictors of brain volumetric measures in schizophrenia patients (n=54)

Model	Change score			Endpoint score		
	R^2	Δ F	*P*	R^2	Δ F	*P*
Cortical Volume						
ICV[a]	0.41	36.3	<0.001*	0.41	36.3	<0.001*
ICV + Demographic[b]	0.52	3.58	0.02*	0.52	3.59	0.02*
ICV + Demographic + Clinical[c]	0.52	0.37	0.55	0.54	2.08	0.16
ICV + Demographic + Clinical + Cognitive[d]	0.63	2.49	0.04*	0.67	6.19	0.001*
White matter volume						
ICV	0.37	30.1	<0.001*	0.37	30.1	<0.001*
ICV + Demographic	0.54	6.15	0.001*	0.54	6.15	0.001*
ICV + Demographic + Clinical	0.62	4.62	0.02*	0.58	2.46	0.09
ICV + Demographic + Clinical + Cognitive	0.64	1.11	0.35	0.06	1.23	0.31
Subcortical Volume						
ICV	0.18	11.5	0.001*	0.18	11.5	0.001*
ICV + Demographic	0.25	1.45	0.24	0.25	1.45	0.24
ICV + Demographic + Clinical	0.25	0.05	0.82	0.32	5.37	0.02*
ICV + Demographic + Clinical + Cognitive	0.30	1.86	0.17	0.47	3.11	0.02*
Cortical Thickness						
Demographic	0.02	0.33	0.80	0.02	0.33	0.80
Demographic + Clinical	N/A			0.09	4.90	0.03*
Demographic + Clinical + Cognitive	N/A			N/A		

[a] ICV= intracranial volume. Cortical thickness not adjusted for ICV

[b] Age, sex, highest level of education achieved.

[c] Clinical factors significant on correlation analysis i.e. For change scores: white matter volume: motor coordination, sensory integration; cortical volume: total insight; subcortical gray: total insight; cortical thickness: nil. For endpoint scores: white matter volume: symptom awareness, sensory integration; cortical volume: symptom awareness, sensory integration; subcortical gray matter volume: sensory integration; cortical thickness: social and occupational functioning.

[d] Cognitive factors significant on correlation analyses i.e. For change scores: white matter volume: processing speed, visual learning, social cognition; cortical volume: processing speed, verbal learning, visual learning, social cognition, working memory; subcortical gray: processing speed, social cognition; cortical thickness: nil. For endpoint scores: white matter volume: reasoning and problem solving, processing speed, working memory, verbal learning,

visual learning, social cognition; cortical gray: working memory, verbal learning; subcortical gray: processing speed, working memory, social cognition, verbal learning; cortical thickness: nil.

Supplementary Table 1: Correlations between brain volume at baseline and clinical and cognitive change scores (state relationships)

	log cortical volume		log white matter volume		log subcortical gray volume		Global Cortical thickness	
Endpoint variables	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
NES								
Total	-.223	p=.113	-.199	p=.157	-.122	p=.389	-0.060	0.665
Sensory integration	-.323	p=.019**	-.340	p=.014**	-.238	p=0.09*	-0.079	0.570
Motor coordination	-.078	p=.581	-.132	p=.350	-.023	p=.873	0.027	0.845
Motor sequencing	-.181	p=.198	-.207	p=.141	-.187	p=.183	-0.030	0.830
MCCB								
Processing speed	.136	p=.337	.298	p=.032**	.283	p=.042**	-0.160	0.248
Attention and vigilance	.061	p=.665	.020	p=.887	.058	p=.685	0.070	0.618
Working memory	.314	p=.023**	.381	p=.005**	.273	p=.050*	-0.044	0.618
Verbal learning	.314	p=.023**	.314	p=.024**	.325	p=.019**	-0.750	0.590
Visual learning	.193	p=.171	.268	p=.054*	.190	p=.179	0.136	0.352
RPS	.174	p=.216	.236	p=.092*	.105	p=.460	0.136	0.352
Social cognition	.182	p=.197	.370	p=.007**	.401	p=.003**	-0.213	0.122
PANSS								
Total	-.151	p=.284	-.090	p=.524	-.001	p=.998	-0.265	0.230
Positive	-.086	p=.546	-.087	p=.540	-.036	p=.800	-0.031	0.840
Negative	-.016	p=.910	.051	p=.721	.090	p=.525	-0.092	0.507
Excitement/hostility	-.118	p=.406	-.095	p=.501	-.104	p=.462	-0.175	0.397
Disorganised	-.215	p=.126	-.188	p=.183	-.123	p=.384	-0.216	0.117
Depression/anxiety	-.192	p=.173	-.156	p=.267	-.064	p=.652	-0.092	0.507
SOFAS	.066	p=.642	-.126	p=.374	-.176	p=.211	0.270	0.048*
CGI	-.031	p=.827	-.019	p=.893	.176	p=.212	-0.099	0.476
CDSS	-.133	p=.349	-.157	p=.267	-.068	p=.632	-0.040	0.773
BIS								
Total	-.156	p=.268	-.248	p=.076*	-.107	p=.451	-0.165	0.230
Symptom attribution	-.047	p=.743	-.011	p=.938	-.139	p=.324	0.028	0.838
Illness awareness	-.268	p=.055*	-.295	p=.034**	-.144	p=.308	0.075	0.588
Need for treatment	.076	p=.593	-.155	p=.274	.111	p=.434	0.086	0.535

NES, Neurological Evaluation Scale; PANSS, Positive and Negative Syndrome Scale; CDSS Calgary Depression Scale for Schizophrenia; SOFAS, Social and Occupational Functioning Assessment Scale; MCCB, MATRICS Consensus Cognitive Battery.

* uncorrected p-value statistically significant at <0.05

** uncorrected p-value<0.1 qualifying for entry into regression model

Supplementary Table 2: Correlations between brain volume at baseline and clinical and cognitive endpoint scores (trait relationships)

	log cortical volume		log white matter volume		log subcortical gray volume		Global Cortical thickness	
Endpoint variables	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
NES								
Total	-.223	p=.113	-.199	p=.157	-.1219	p=.389	-0.060	0.665
Sensory integration	-.323	p=.019**	-.340	p=.014**	-.238	p=0.09*	-0.079	0.570
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Motor sequencing	-.181	p=.198	-.207	p=.141	-.187	p=.183	-0.030	0.830
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NES, Neurological Evaluation Scale; PANSS, Positive and Negative Syndrome Scale; CDSS, Calgary Depression Scale for Schizophrenia; SOFAS, Social and Occupational Functioning
Assessment Scale; MCCB, MATRICS Consensus Cognitive Battery.

* uncorrected p-value statistically significant at <0.05

** uncorrected p-value<0.1 qualifying for entry into regression model

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CHAPTER 6

BRAIN VOLUME CHANGES OVER THE FIRST YEAR OF TREATMENT IN SCHIZOPHRENIA: RELATIONSHIPS TO ANTIPSYCHOTIC TREATMENT

Published, second author:

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ORIGINAL ARTICLE

Brain volume changes over the first year of treatment in schizophrenia: relationships to antipsychotic treatment

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Background. Progressive brain volume reductions have been described in schizophrenia, and an association with antipsychotic exposure has been reported.

Methods. We compared percentage changes in grey and white matter volume from baseline to month 12 in 23 previously antipsychotic-naïve patients with a first episode of schizophrenia or schizophreniform disorder who were treated with the lowest effective dose of flupenthixol decanoate depot formulation, with 53 matched healthy individuals. Total antipsychotic dose was precisely calculated and its relationship with brain volume changes investigated. Relationships between volumetric changes and treatment were further investigated in terms of treatment response (changes in psychopathology and functionality) and treatment-related adverse-events (extrapyramidal symptoms and weight gain).

Results. Excessive cortical volume reductions were observed in patients [−4.6 (6.6)%] *v.* controls [−1.12 (4.0)%] ($p = 0.009$), with no significant group differences for changes in subcortical grey matter and white matter volumes. In a multiple regression model, the only significant predictor of cortical volume change was total antipsychotic dose received ($p = 0.04$). Cortical volume change was not significantly associated with the changes in psychopathology, functionality, extrapyramidal symptoms and body mass index or age, gender and duration of untreated psychosis.

Conclusions. Brain volume reductions associated with antipsychotic treatment are not restricted to poor outcome patients and occur even with the lowest effective dose of antipsychotic. The lack of an association with poor treatment response or treatment-related adverse effects counts against cortical volume reductions reflecting neurotoxicity, at least in the short term. On the other hand, the volume reductions were not linked to the therapeutic benefits of antipsychotics.

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Key words: Brain-volume, cortex-volume, schizophrenia, antipsychotic, flupenthixol, dose-response.

Introduction

Imaging studies have consistently demonstrated differences in brain morphology in people with schizophrenia compared with healthy controls (Haijma *et al.* 2013). The evolution of these abnormalities over the course of illness has been a subject of interest. Smaller grey matter volumes are apparent in the prodrome, and additional reductions have been reported in the months immediately after onset of first psychotic symptoms (Pantelis *et al.* 2003). Furthermore, longer term studies indicate that progressive reductions in brain volume continue to occur (Hulshoff Pol & Kahn, 2008; Smieskova *et al.* 2009; Kempton *et al.* 2010; Olabi *et al.* 2011). While these progressive changes have been thought to reflect disease progression (Lieberman *et al.* 2001), recent attention has focused on the possibility that they may, at least in part, be related to antipsychotic treatment. Attention was drawn to this possibility when it was reported that macaque monkeys exposed to haloperidol or olanzapine over 17–27 months showed substantial brain volume reductions (Dorph-Petersen *et al.* 2005). A few studies have directly investigated this possibility. In a study in patients with schizophrenia who underwent repeated neuroimaging for up to 14 years, greater intensity of antipsychotic treatment was associated with smaller grey matter volumes and progressive reductions in white matter volume, while illness severity had relatively modest correlations with volume reductions. These authors concluded that antipsychotics have a subtle but measurable influence on brain tissue loss over time (Ho *et al.* 2011). In a study of 33 patients with schizophrenia and 71 controls selected from the Northern Finland Birth Cohort 1966 who underwent a magnetic resonance imaging (MRI) brain scan at the age of 33–35 years and a follow-up scan 9 years later the mean annual whole-brain volume

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reduction was 0.69% in schizophrenia, and 0.49% in controls ($p = 0.003$). The volume reductions were not associated with symptom severity, functionality or cognitive decline, but the estimated amount of antipsychotic medication prescribed over the follow-up period predicted brain volume loss ($p = 0.003$) (Veijola *et al.* 2014). Finally, a subsequent voxel-wise analyses of the same Northern Finland Birth Cohort sample found that, compared with controls, patients exhibited greater progressive brain reductions, and that ventricular enlargement was predicted by greater exposure to antipsychotic medication (Guo *et al.* 2015).

The relationship between brain volume changes and antipsychotic treatment in schizophrenia has also been addressed retrospectively, in several systematic reviews and meta-analyses. An earlier review of structural MRI studies reported that first-generation antipsychotics (FGAs) were associated with increased basal ganglia volume and second-generation antipsychotics (SGAs) with decreased volume of the thalamus. Results also suggested an effect on the cortex with FGAs being associated with volume reductions, possibly with a dose-dependent effect, and SGAs with retention or increase in grey matter volumes (Navari & Dazzan, 2009). A systematic review and meta-analysis conducted in over 18 000 patients reported that, compared with healthy controls, brain volume was significantly smaller in patients with schizophrenia. Differences were most pronounced for grey matter and were associated with a higher prescribed dose of antipsychotic medication (Haijma *et al.* 2013). In a meta-analysis specifically investigating the relationship between longitudinal brain changes and antipsychotic treatment, progressive grey matter volume reductions and lateral ventricular enlargements were found in patients but not in controls. Grey matter volume changes were inversely correlated with estimated cumulative exposure to antipsychotics (Fusar-Poli *et al.* 2013). A recent meta-analysis assessing the association between loss of cortical grey matter volume and cumulative antipsychotic intake found that the effect was more marked in patients who had been treated with at least one FGA (Vita *et al.* 2015). Findings to date need to be interpreted with caution due to important methodological limitations (Fusar-Poli *et al.* 2013; Guo *et al.* 2015). Most studies were retrospective and conducted in naturalistic settings; estimates of treatment exposure were based on chart reviews; patients had been exposed to varying amounts of antipsychotic medication prior to baseline assessments; they received different antipsychotics over varying follow-up periods; the actual amount of medication received was not systematically documented and adherence was not effectively assessed. An additional potential confound is illness severity, although one study found only weak correlations (Ho *et al.* 2011) between symptom severity and brain volume change and another failed to find a relationship (Veijola *et al.* 2014). Patients with more severe illness are likely to receive higher doses of antipsychotics, so that an association between dose and brain volume reduction may actually reflect illness severity rather than an effect of the medication.

Finally, not all studies have reported progressive brain volume reductions. Indeed, it has been argued that deterioration in outcome over time does not equate with neuroprogression, but may rather be a consequence of secondary factors such as poor access or adherence to treatment, the effects of concurrent conditions and social and financial impoverishments (Zipursky *et al.* 2013). In a recent 1-year follow-up study, patients with first-episode psychosis did not differ significantly from controls in annual percentage change in cortical thickness or subcortical structures, and antipsychotic use was not related to longitudinal brain change (Haukvik *et al.* 2016). Also, another systematic review of longitudinal MRI studies did not find a consistent relationship between antipsychotic treatment exposure and brain changes over time (Roiz-Santianez *et al.* 2015).

In this study, we investigated brain volume changes during the first 12 months of standardised treatment in a carefully selected sample of previously never-treated individuals with schizophrenia. We also assessed the relationship between brain volume changes and antipsychotic treatment in some detail. Antipsychotic treatment effect was considered in terms of treatment response (changes in psychopathology and functionality), total antipsychotic dose and treatment-related adverse effects (weight gain and extrapyramidal symptoms). We were able to address many of the potentially confounding factors from previous studies. By including only antipsychotic naïve patients we eliminated potential effects of the previous treatment. This may be important, given that onset of antipsychotic action is rapid (Kapur *et al.* 2005), and striatal grey matter volume reductions were demonstrated in healthy volunteers within hours after a single dose of haloperidol (Tost *et al.* 2010). Also, by selecting first-episode patients, we avoided effects of disease chronicity. Further, because our patients were treated according to a standard protocol, largely with a single antipsychotic and as a long acting injectable formulation, we were able to calculate precisely the total 12-month antipsychotic dose that each individual received. Regular follow-up assessments during the 12-month treatment period allowed us to assess changes in psychopathology, functionality and treatment-related adverse effects of weight-gain and extrapyramidal symptoms. The aims of the study were to determine whether changes in cerebral grey and white matter volume occur during the first year of antipsychotic

treatment, and if so, whether such changes are related to antipsychotic treatment. We hypothesised that, compared with controls, patients would exhibit excessive grey matter volume reductions, and that these reductions would be associated with greater cumulative antipsychotic dose, poorer treatment response, emergent extrapyramidal symptoms and weight gain.

Methods and materials

This was an open-label, longitudinal study in which patients were treated for 12 months with flupenthixol decanoate and clinical and biological correlates of outcome assessed. We obtained ethics approval from the Human Research Ethics Committee of Stellenbosch University. The study was conducted according to the International Conference on Harmonization good clinical practice guidelines (International Conference on Harmonization, 1996).

Participants

Patients were recruited from psychiatric hospitals and clinics in Cape Town and surrounds between April 2007 and March 2011. They were all voluntary patients, and provided written, informed consent to participate in the study. In the case of minors, we obtained consent from the legal guardian. Inclusion criteria for the parent study were men and women, aged 16–45 years, experiencing a first psychotic episode meeting Diagnostic and Statistical Manual of Mental Diseases, Fourth Edition, Text Revisions (DSM-IV TR) (American Psychiatric Association, 1994) diagnostic criteria for schizophrenia or schizophreniform disorder. Exclusion criteria were serious or unstable general medical condition, current substance abuse and an educational level <Grade 7. For the present study we only included patients who had never been exposed to antipsychotic medication and who had both baseline and month 12 scans. Of 93 patients who underwent baseline scans, 70 were excluded [no month 12 scans due to study discontinuation or non-availability of the scanner ($n = 44$), previous antipsychotic exposure ($n = 16$) and poor scan quality ($n = 11$)]. Thus, 23 antipsychotic-naïve patients with baseline and 12-month scans who had adhered to the treatment protocol were included. The included patients did not differ significantly from the excluded patients regarding age, gender, ethnicity, highest level of schooling, DSM-IV TR diagnosis and baseline the Positive and Negative Syndrome Scale (PANSS) total scores, but the included patients had significantly higher baseline Social and Occupational Functioning Assessment Scale (SOFAS) scores [50(14) *v.* 43(10), $p = 0.006$]. Healthy controls, matched (not individually) in the parent study by age, sex, ethnicity and educational status were solicited through personal contacts and advertisements from non-medical hospital staff, relatives, acquaintances and from independent sources in the community. They were excluded if they reported a history of mental illness, previous treatment with psychotropic medication or substance abuse. Of 104 controls 51 were excluded [no month 12 scans ($n = 41$), poor quality scans (n = 10)], leaving a healthy control group of 53.

Treatment

Patients were treated according to a fixed protocol, with depot antipsychotic. Depot formulation provides assured medication delivery and is increasingly considered as an early treatment option where the benefits of improved adherence may be greatest (Brissos *et al.* 2014). We chose flupenthixol decanoate as it is widely available, affordable and remains a popular choice of psychiatrists for treating psychosis (Shen *et al.* 2012). Flupenthixol is a high potency thioxanthene, whose receptor binding profile of D1–5 dopamine, 5-HT2, H1 histamine and α−1 adrenergic-antagonism is not dissimilar to several SGAs (de Wit, 2010). There was a week lead-in with oral flupenthixol 1–3 mg/day followed by flupenthixol decanoate intramuscular injections 2-weekly for the study duration. The initiation dose was 10 mg 2-weekly. Additional oral flupenthixol was permitted, but seldom prescribed. Flupenthixol decanoate was maintained at the lowest possible dose, only increased when insufficient response persisted. For initial agitation lorazepam was prescribed, rather than increasing the antipsychotic dose. Permitted concomitant medications included lorazepam, anticholinergics, propranolol, antidepressants and medications for general medical conditions. No benzodiazepines, propranolol or anticholinergics were permitted in the 12 h before assessments. Prohibited medications included other antipsychotics, mood stabilisers and psychostimulants. Six participants were treated with long-acting risperidone injection for the first 12 weeks of the study, before being switched to flupenthixol decanoate. For these patients there was a week lead-in of oral risperidone, continued for 3 weeks. The starting dose for long-acting risperidone was 25 mg IMI 2-weekly. Using depot antipsychotic meant that the potentially confounding effect of covert non-adherence was removed. It also allowed us to accurately calculate the total dose of antipsychotic that each patient received over the 12 months of treatment. We calculated dose equivalencies according to consensus-derived guidelines (Gardner *et al.* 2010) and the total study antipsychotic dose was expressed as flupenthixol decanoate mg equivalents.

Assessments

Patients and controls were assessed with the SCID (Structured Clinical Interview for DSM-IV) (First *et al.* 1994). Patients were assessed three-monthly with the PANSS (Kay *et al.* 1987), the SOFAS (American Psychiatric Association, 1994) and the Extrapyramidal Symptom Rating Scale (ESRS) (Chouinard & Margolese, 2005). Investigators underwent training and inter-rater reliability (IRR) testing. The IRR was >75% for all scales. For body-mass measurements patients removed surplus clothing and were weighed on a calibrated electronic scale. Height was measured with a prefixed, wall-mounted measuring tape.

Imaging methods

Patients underwent baseline scans before receiving any antipsychotic medication. We acquired high-resolution T1-weighted data on a 3 T Siemens Allegra MRI scanner (Erlangen, Germany) with the following acquisition parameters: MPRAGE sequence, 2080 ms repetition time; 4.88 ms echo-time, Field of view: 230 mm, 176 slices, $0.9 \times 0.9 \times 1$ mm^3 voxel size. All scans were screened for intracranial pathology and motion artefacts. Scans were processed using Freesurfer version 5.1. (http://surfer.nmr.mgh.harvard.edu/) (Dale *et al.* 1999; Fischl *et al.* 1999). Slices were resampled to a three-dimensional image with 1 mm isotropic voxels. Non-uniform intensity normalisation was performed and images registered to the Montreal Neurological Institute space. A second normalisation step was performed with control points automatically identified and normalised to a standard intensity value, followed by an automated skull strip procedure. Gross brain anatomy was delineated into cortical and subcortical labels. Reconstructions were performed with custom batching scripts, on the Centre for High Performance Computing, Cape Town, Sun Intel Nehalem cluster (http://www.chpc.ac.za/). All data were visually inspected for errors in Talairach transformation, skull strip, final segmentations and within subject-registrations. Errors were corrected manually and re-inspected.

Measures of brain volume

We selected the following global measures of brain volume: (1) Cortical volume – a surface-based volume calculation, comprising the volume inside the pial surface minus the volume inside the white surface minus tissue inside the ribbon that is not part of cortex. (2) Subcortical grey matter volume – a voxel count of the thalamus, caudate, hippocampus, amygdala, accumbens, ventral diencephalon and substantia nigra. (3) White matter volume – using surface-based volume computation for part of the calculation and voxel counts to subtract anything that is not white matter.

Table 1. *Baseline characteristics for the 23 patients and 53 controls*

	Patients	Controls	*p*
Age, years, mean (S.D.)	25.26 (6.5)	27.20 (7.7)	0.3
Males, *n* (%)	18 (78%)	32 (60%)	0.1
Ethnicity *n* (%)			
Mixed ancestry	17 (74%)	40 (76%)	0.9
Black	3 (13%)	7 (13%)	
White	3 (13%)	6 (11%)	
Highest school grade passed in years, mean (S.D.)	10.3 (2)	10.9 (1.4)	0.2
DSM–IV TR diagnosis, *n* (%)			
Schizophrenia	16 (70%)		
Schizophreniform	7 (30%)		
DUP, weeks, mean (S.D.)	45.3 (48.3)		

Statistical methods

Normality of data distribution was assessed by histograms and differences in demographic and clinical characteristics between patients and controls compared by two-sample *t*-tests and χ^2 tests for continuous and categorical variables, respectively. All tests were two-tailed. We used analysis of covariance to compare groups for percentage volume change for the three brain volumetric measures, with intracranial volume as covariate. Bonferroni corrections were applied for multiple comparisons and the adjusted significance level was set at 0.017. To investigate whether significant brain volume changes were predicted by changes in clinical and treatment-related variables we used linear regression with percentage brain volume change as the dependent variable, gender as a factor and the following continuous predictors: Change from baseline to month 12 in PANSS total score, SOFAS and body mass index (BMI); change from baseline to maximum score for ESRS total; total calculated 12-month antipsychotic dose; age and duration of untreated psychosis (DUP). In view of the small sample, we used best subsets regression and restricted the number of variables in the model to four. Analyses were performed using Statistica 13 software (Dell).

Results

Baseline characteristics for the patients and controls are provided in Table 1. Baseline, month 12 and % change values for clinical and brain volumetric measures are provided in Table 2. The mean (S.D.) total 12-month dose of flupenthixol decanoate equivalents was 312.4 (100.6) mg. The minimum dose was 130.1 mg and the

Table 2. *Baseline and month 12 values for the clinical and brain volume measures*

	Patients ($n = 23$)			Controls ($n = 53$)				
	Baseline	Month 12	% change	Baseline	Month 12	% change	t*	p*
PANSS total score, mean (S.D.)	89.3 (48.3)	41.3 (12)	−46.2 (15.8)					
SOFAS score, mean (S.D.)	50.5 (14.4)	68.0 (13.7)	17.5 (13.7)					
ESRS total score, mean (S.D.)	0.4 (1.2)		4.1 (3.5)					
BMI (kg/m^2), mean (S.D.)	21.2 (3.22)	24.5 (4.6)	3.2 (2.5)					
Cortical grey matter volume (mm^3), mean (S.D.)	449 949 (57 281)	428 394 (55 739)	−4.6 (6.6)	445 567 (57 113)	439 688 (51 724)	−1.12 (4.0)	−2.8	0.006
Total white matter volume (mm^3), mean (S.D.)	492 096 (66 458)	503 183 (65 393)	2.5 (6.0)	504 577 (61 598)	511 750 (67 154)	1.40 (4.2)	0.9	0.4
Subcortical grey matter volume (mm^3), mean (S.D.)	188 398 (22 546)	189 056 (23 078)	0.5 (5.4)	188 482 (23 379)	190 586 (22 887)	1.50 (8.2)	−0.6	0.6

*t-tests for % change in volume, patients *v.* controls.

maximum dose was 495.3 mg. Ten (43%) of the patients received anticholinergics for a mean (S.D.) of 7.7 (13.0) weeks; 7 (30%) received antidepressants for 7.1 (13.8) weeks; and 13 (57%) received benzodiazepines for 4.3 (10.1) weeks. Patients and controls did not differ significantly regarding age, sex, ethnicity and highest school grade passed and there were no group differences for the brain volumetric measures at baseline.

MRI volumetric changes

Percentage change from baseline to month 12 for the three brain volume measurements is provided in Fig. 1. After co-varying for intracranial volume there were significantly greater reductions in cortical volume in patients *v.* controls (F 7.3, $p = 0.009$), but no significant differences for changes in subcortical grey matter ($F = 0.4$, $p = 0.5$) and white matter volume ($F = 1.0$, $p = 0.3$).

Predictors of volume changes

For the regression analysis with % change in cortical volume as the dependent variable a model, including age, total antipsychotic dose, SOFAS change score and ESRS change score explained 31% of the variance ($F = 2.0$, $p = 0.1$) and the only variable to significantly predict cortical volume change was total antipsychotic dose received ($F = 5$, $p = 0.04$). Change in cortical volume was not significantly associated with age, gender, DUP or changes in PANSS total score, SOFAS score, BMI and ESRS.

Discussion

This study indicates that, compared with healthy controls, previously unmedicated patients with schizophrenia displayed excessive reductions in cortical grey matter volume after 12 months of antipsychotic treatment. Reductions were predicted by total antipsychotic dose but not by treatment response or treatment-related adverse effects. Specifically, we found no significant associations between cortical volume reduction and changes in psychopathology, functionality, extrapyramidal symptoms and BMI, or age, gender and DUP. These findings are remarkable insofar as the volume reductions occurred in patients who had generally responded favourably to treatment [19 (83%) achieved operationally defined remission (Andreasen *et al.* 2005)] and had received the lowest effective dose of antipsychotic [mean endpoint dose flupenthixol decanoate 11.1(6.1) mg 2-weekly]. While these findings are in contrast to the reported association between brain volume reductions and poorer outcome (Lieberman *et al.* 2001), our sample was different in that we only included patients who completed 12 months of treatment, and excluded non-responders.

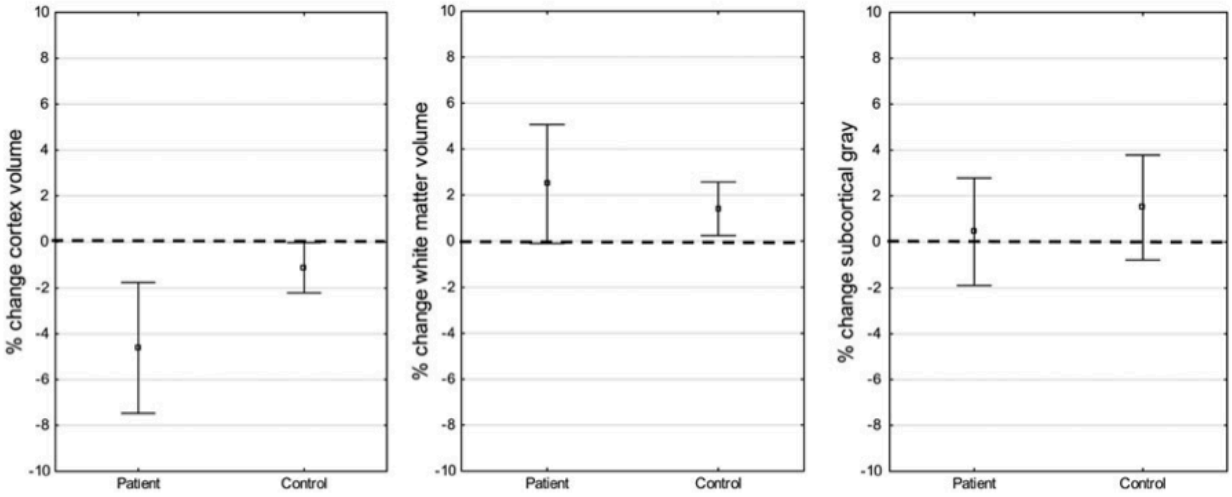

Fig. 1. Percentage change in brain volumes from baseline to month 12 for the patients and controls (means and 95% confidence intervals).

It could be that even greater volume reductions occur in non-responders. In any event, our findings suggest that brain volume reductions are not restricted to those patients with a poor treatment outcome. It is important to keep in mind, however, that the previous study reporting an association between brain volume reduction and poor outcome (Lieberman *et al.* 2001) did not control for antipsychotic exposure, and the current study adds to evidence suggesting that the findings of that study were confounded by this factor.

The −4.6% cortical volume reduction is considerable, given the relatively brief treatment period of 12 months. While this reduction appears at first sight to be greater than the mean estimated annual whole-brain volume reduction of −0.69% in schizophrenia (Veijola *et al.* 2014) and the −2.6% total brain volume reduction reported in a meta-analysis of medicated patients with schizophrenia, the former analysis was not conducted on first-episode patients and the latter analysis actually found a reduction in total grey matter of −4.3%, with no significant white matter reductions (Haijma *et al.* 2013) – i.e. results that are similar to ours. The pronounced volume reduction in our sample is consistent with a more marked effect in the early course of illness (Andreasen *et al.* 2011), and suggests that critical brain structural changes may take place in the period immediately following the first-onset of psychosis (Pantelis *et al.* 2003). The volume reduction that we found is however less than the 8–11% brain-weight and volume reduction reported in macaque monkeys over 17–27 months of antipsychotic treatment at plasma drug levels similar to those in treated humans (Dorph-Petersen *et al.* 2005). Also, the absence of significant white matter reductions in our study differs from the findings in that study where both grey and white matter reductions were reported (Dorph-Petersen *et al.* 2005). Conversely, others have found, as in our study, that volume reductions associated with antipsychotic treatment to be restricted to grey matter (Fusar-Poli *et al.* 2013). Furthermore, while Ho *et al.* (2011) reported progressive volume reductions in both grey and white matter that were most evident in patients who received more antipsychotic treatment, an earlier study of theirs found that antipsychotic dose was related to the rate of loss of frontal grey matter, but only in patients who were medication naïve at baseline (Ho *et al.* 2007). Therefore, it may be that antipsychotics contribute to grey matter loss in the early course of treatment, and later to white matter loss (Goff, 2011).

Our failure to find significantly different changes in subcortical grey matter volume in patients *v.* controls differs from previous reports of increased basal ganglia volume associated with FGAs and increased thalamic volume associated with SGAs (Dazzan *et al.* 2005). On the other hand, our findings are consistent with a later systematic review of volumetric changes in basal ganglia after antipsychotic monotherapy that found no support for basal ganglia volume increases with FGAs, and both volumetric increases and decreases being reported with SGAs (Ebdrup *et al.* 2013).

Nature of the relationship between brain volume reduction and antipsychotic treatment:

Antipsychotic treatment may be related to brain volume changes in several ways. First, it may be linked to efficacy – or the lack thereof – of medication. Regarding the former, the possibility has been raised that brain volume reductions are related to the same mechanism that provides the therapeutic effect of

antipsychotics (Lewis, 2011). If that were the case, an association between brain volume reduction and symptom improvement would be expected. Our study failed to demonstrate any such relationship, thereby counting against brain volume changes being linked to the mechanism responsible for antipsychotic efficacy. Similarly, if the brain volume reductions are associated with antipsychotic neurotoxicity and poor treatment response (Lieberman *et al.* 2001) a negative correlation between brain volume reduction and symptom reduction would be anticipated. Again, our findings do not support this possibility. It remains feasible, however, that antipsychotics are effective in treating symptoms of psychosis in the short term, but are responsible for neuroprogressive changes in the longer term.

Another possibility is that the association between brain volume change and antipsychotic treatment is linked to treatment-related side-effects. Indeed, acute treatment with haloperidol has been associated with striatal volume reductions, which strongly predicted the development of extrapyramidal symptoms (Tost *et al.* 2010), and in people with schizophrenia more pronounced brain volume reductions have been reported in those with tardive dyskinesia compared with those without tardive dyskinesia (Sarro *et al.* 2013). We found no evidence of an association between brain volume changes and extrapyramidal symptoms. However, an association cannot be ruled out as extrapyramidal symptoms were generally very mild in our sample and none of our patients developed persistent dyskinesia. Another possibility is that brain volume loss is related to the adipogenic effects of antipsychotics. It is well recognised that obesity is associated with cortical thinning (Medic *et al.* 2016) and treatment-naïve first-episode patients are particularly susceptible to the weight-gain effects of antipsychotics (Strassnig *et al.* 2007). Furthermore, we previously reported ventral diencephalon volume reductions, which were significantly correlated with BMI increase during the first 12 weeks of antipsychotic treatment in never-treated first-episode schizophrenia patients (Emsley *et al.* 2015). However, while the mean increase in BMI of 3.2 kg/m^2 over 12 months in our cohort was substantial, we were unable to demonstrate a link between brain volume changes and weight-gain.

Regarding the underlying neurobiology, there are several possible explanations for the observed cortical volume reductions in our study. Besides the possibility of neuronal damage they could reflect structural plasticity involving remodelling of neuronal processes, changes in water content, or a decrease in the number of non-neuronal cells (Zatorre *et al.* 2012). Also, cortical volume reduction could be related to the reported anti-inflammatory effect of antipsychotics (Al-Amin *et al.* 2013). Neuroinflammation increases local blood flow and vascular permeability, cytokine production, activation of microglia and infiltration of mobile cells of the immune system (Graeber *et al.* 2011). An anti-inflammatory effect of antipsychotics would be expected to reverse these changes, which may result in volume reductions.

Dose–response relationship

An important aspect of this study is that we were able to accurately calculate the total dose of antipsychotic that each patient received. Patients had never been exposed to any antipsychotic previously; they received a single antipsychotic (except for six patients who received long-acting risperidone for the first 12 weeks); and using a depot formulation meant that the amount prescribed was the amount received. This enabled us to avoid shortcomings of previous studies where multiple antipsychotics were prescribed, and where covert partial and non-adherence are likely to have played a role. The role of non-adherence as a potential confounder should not be underestimated, particularly for first-episode schizophrenia, where non- and partial-adherence rates are very high (Coldham *et al.* 2002). Patients who are not fully adherent do not respond optimally, and clinicians are likely to respond by increasing the antipsychotic dose. Thus, a dose–response effect could alternatively reflect volume reductions associated with persistence of psychotic symptoms due to non-adherence rather than a direct effect of treatment. Thus, the finding of a significant relationship between antipsychotic dose and cortical volume reduction in this study strongly suggests causality.

Strengths and limitations

Strengths of the study include the careful selection of the sample (antipsychotic naïve, first-episode); standardisation of treatment with a single antipsychotic and a low-dosing strategy; using depot formulation, which allowed precise calculation of the total antipsychotic dose and avoided confounding effects of covert non-adherence; regular assessment of treatment response in terms of psychopathology and functionality and adverse effects in terms of weight change and emergent extrapyramidal symptoms. The study is limited by the small sample and was not sufficiently powered to explore regional differences in brain volume change. Also, the inclusion age range of 16–45 years was arguably too wide for such a small sample. Further, the limited follow-up period of 12 months precludes any inferences on possible longer term effects of antipsychotics on brain volume. Finally, our findings

cannot necessarily be generalised to other antipsychotics, particularly in the light of reports of class differences in brain volume changes (Navari & Dazzan, 2009; Vita *et al.* 2015). However, we consider it unlikely that our findings are restricted to treatment with FGAs, for the following reasons: FGAs and SGAs are not homogeneous classes and it has been recommended that this distinction be abandoned (Leucht *et al.* 2009); early studies with FGAs may have been biased by the use of excessively high doses (Geddes *et al.* 2000); brain-weight and volume reductions reported in antipsychotic-treated macaque monkeys were of similar magnitude for a SGA (olanzapine) and FGA (haloperidol) (Dorph-Petersen *et al.* 2005); and flupenthixol is considered a 'partially atypical' antipsychotic (Gattaz *et al.* 2004), sharing receptor binding characteristics of several SGAs (de Wit, 2010).

Conclusion

Morphological brain changes associated with antipsychotic treatment are not restricted to patients with a poor treatment outcome, and occur even with the lowest effective dose. Cortical volume reductions do not appear to be associated with illness progression or adverse effects that may reflect neurotoxicity, at least in the short term. At the same time we found no evidence to link volume reductions with the therapeutic benefits of antipsychotics.

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Declaration of Interest

Robin Emsley has received honoraria from Janssen, Lundbeck, Servier and Otsuka for participating in advisory boards and speaking at educational meetings, and has received research funding from Janssen and Lundbeck. Bonginkosi Chiliza has received honoraria from Lundbeck and Janssen for speaking at educational meetings. All of the other authors have declared that there are no conflicts of interest in relation to the subject of this study.

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CHAPTER 7: CONCLUSION

This chapter synthesises the findings of the included journal manuscripts, summarizes contributions to existing knowledge, and ends with a section on future research directions. The PhD comprises five journal articles each asking distinct neuroimaging research questions that are connected in terms of clinical relevance at a local and international level.

The focus of Chapter 2 and 3 is on brain deficits associated with impaired insight in FES. Impaired insight is a highly prevalent feature of schizophrenia that is associated with poorer outcome (Margariti et al. 2015; Yalcin-Siedentopf et al. 2014; Johnson et al. 2012; Amador et al. 1994). Poor treatment adherence and the related issue of diminished insight in schizophrenia was fundamental to the study design of the parent study where we combined the lowest effective dose of flupenthixol decanoate medication with an assertive monitoring program (Chiliza et al. 2016). For my PhD, we examined the relationship between insight, as measured by the Birchwood Insight Scale (BIS) (Birchwood et al. 1994), and brain deficits using two neuroimaging modalities, namely diffusion tensor imaging (chapter 2) and cortical thickness (chapter 3).

In Chapter 2, we examined for an association between white matter fractional anisotropy and clinical insight in 89 FES patients. Looking at other neuroimaging studies, evidence for clear patterns in relation to insight in schizophrenia is limited. This might be because insight is a nuanced, multifaceted concept and may fractionate across clinical and cognitive dimensions into a number of domains (David et al. 2012). Indeed, we found that the white matter neural signature of total insight was widespread and predicted by a complex network of tracts. On the other hand, we found that the network of tracts that predicted the domains of 'illness awareness' and 'need for treatment' were separable, and that FA values did not predict the 'symptom attribution' domain.

Shad and Keshavan (Shad and Keshavan 2015) hypothesised that fewer and more specific frontal areas may be involved in symptom misattribution, possibly due to a failure to recruit compensatory brain mechanisms. We therefore extended their theory in Chapter 3 by specifically examining the relationship between symptom attribution and frontal cortical thickness in 92 FES patients. Symptom misattribution was associated with significantly thinner left anterior cingulate and left rostral middle frontal cortices. Symptom attribution, which involves the patient's ability to relabel the signs and symptoms of schizophrenia as pathological, is thought to require a higher level of insight functioning than the other clinical insight domains, in that it involves the ability to monitor and control ongoing mental activities (David et al. 2012). The rostral middle frontal region is akin to the dorsolateral prefrontal cortex and plays an important role in executive function, concept flexibility and self-monitoring. These functions are central to the ability to properly recognize, interpret and

relabel symptoms and signs (Morgan et al. 2010). Also in keeping with the metacognitive nature of symptom attribution (David et al. 2012), the anterior cingulate has been found to play a key role in the aberrant attribution of salience to stimuli in schizophrenia (Palaniyappan and Liddle 2012), and as a result may contribute to the incorrect attribution of symptoms in the illness.

An additional finding from our neuroimaging insight studies is that patients who were more depressed had significantly greater total insight, increased recognition of the need for treatment and increased illness awareness (detailed in Chapter 2). Meta-analyses of studies examining the relationship between depression and insight corroborate these findings (Murri et al. 2015; Mintz et al. 2003). We did not find a relationship between insight and positive and negative symptomatology which is unsurprising given that results from other studies are inconsistent and correlations when found are at best modest (Mintz et al. 2003). A recent commentary recommended that future research in the neurobiology of insight in schizophrenia should be in larger samples in the early phase of the illness to avoid the confounding effects of medication and illness chronicity, and should address different dimensions of insight (Shad 2017). Our studies go some way in addressing this need. Our team is now in the process of analysing the longitudinal insight data from the parent study. Also, in the next cohort of schizophrenia patients that we recruited, we included an assessment of cognitive insight.

As a first step in the methodology of the DTI insight journal article in Chapter 2, we examined for FA differences between 89 FES patients and 98 matched healthy controls. We found widespread reduction in FA in FES patients compared to controls and our subgroup analysis found no difference in FA between minimally treated and treatment naïve patients, but clear differences between patients and controls. DTI findings in FES are variable (Wheeler and Voineskos 2014). At the time of manuscript publication, this was the largest known DTI cohort of FES patients. Again, the main advantages of our cohort are the large sample size in a difficult to recruit population, and the ruling out of long-term medication and chronicity as far as possible as confounds. Our findings therefore lend considerable support to the theory that white matter changes in schizophrenia are an intrinsic marker of the disorder (Haijma et al. 2012), and appear to be linked to the neurodevelopmental model of the illness (Sommer and Kahn 2014; Collin et al. 2013).

There is growing evidence that childhood and adolescence are sensitive stress exposure periods when structures and pathways impacted by trauma are most vulnerable. This can result in an alteration in trajectories of brain development and childhood trauma is a risk factor for schizophrenia. Given that levels of childhood trauma are higher in Low and Middle-

Income Countries (LMICs) compared to High Income Countries (Viola et al. 2016), it is especially important that LMICs are at the forefront of childhood trauma research. We have reported in an earlier study, that the rates of childhood trauma in both patients and controls in our cohort were considerably higher than those reported in other studies (Kilian et al. 2017). Given this concerning finding and its possible impact on neurodevelopment, we examined the relationship between childhood trauma and FA in 53 FES patients and 51 controls. Our findings highlight altered cortico-limbic circuitry in FES patients compared to controls and differential effects of childhood emotional neglect (increased FA) and sexual abuse (decreased FA) on white matter in patients. To our knowledge, at the time of journal manuscript submission for publication, this was the first study examining the relationship between childhood trauma, FA and FES.

Examining for neuroimaging predictors of symptom expression in FES studies has been met with limited success. In Chapter 5, we carefully investigated the relationship between broad neuroimaging measures (global cortical thickness and global cortical, subcortical and white matter volume) and a wide range of trait and state clinical and cognitive symptoms. Our results suggest a differential association between state and trait symptom expression and global brain measures, in that baseline subcortical gray matter volume was significantly associated with sensory integration and verbal learning trait scores, cortical volume with verbal learning trait scores, cortical thickness with social and occupational functioning trait scores, and white matter volume with motor coordination state scores. Of particular potential relevance to patient care, is that these neuroimaging deficits noted at initial presentation in FES, may predict enduring trait deficits in cognition, functioning and neurological soft signs.

In Chapter 6, we found substantial cortical brain volume reductions over twelve months of treatment in antipsychotic naïve patients compared to matched controls. Total antipsychotic dose was a predictor of this reduction in patients however cortical reduction occurred at even the lowest effective antipsychotic dose and unrelated to treatment related side effects or treatment outcome. Although previous longitudinal neuroimaging studies have described progressive reduction in brain volume in schizophrenia (Liberg et al. 2016; Hulshoff Pol and Kahn 2008; Pantelis et al. 2005), and that these progressive changes may at least in part be due to antipsychotic treatment (Vita et al. 2015; Lieberman et al. 2005), most prior studies were retrospective and antipsychotic medication type, dose and adherence was not systematically assessed. In our study, all patients were on a single antipsychotic type and the depot formulation ensured that dose received and adherence was known. Our study contributes significantly to this knowledge field in that our finding of a significant relationship between antipsychotic dose and cortical volume reduction in this study strongly suggests causality.

LOCALLY AND GLOBALLY RELEVANT FUTURE RESEARCH DIRECTIONS

Clinically nested neuroimaging research is dependent on the validity of the available clinical data. Therefore, for the next phase of our study, we have strengthened our assessment of 'insight' by including an assessment of cognitive insight (Beck et al. 2004). Further, our schizophrenia team was recently trained by Prof Helen Fisher, an expert in childhood trauma, in the Childhood Experience of Care and Abuse Interview (CECA) a comprehensive structured narrative interview that assesses childhood trauma retrospectively (Bifulco et al. 2005). Prof Paola Dazzan also hosted a workshop on clinical outcomes in schizophrenia and the clinical utility of neuroimaging in psychotic disorders. Both researchers are from the Institute of Psychiatry, Psychology and Neuroscience, King's College London and we hope to develop this collaboration further.

Two papers in this PHD are related to DTI. The focus has shifted internationally and is evolving in our own unit towards investigating connections across and within networks rather than exclusively examining DTI measures along discrete pathways (Collin et al. 2016). There is increasing evidence that the manifestations of schizophrenia are not solely related to disturbances of individual brain regions but emerge from their interaction within local networks as well as within multiple, large-scale neural systems (Narr and Leaver 2015). Members of our neuroimaging team are currently being trained in connectomics and the related advanced computational analysis with a particular focus on the clinical utility of the connectome. We hope to look at childhood trauma and its relationship to the connectome in schizophrenia and healthy controls as a potential study.

This PhD highlighted frequent inconsistencies in neuroimaging studies and one potential reason for these inconsistencies is that single modality studies may only partially detect neurobiological deficits (Sui et al. 2012). We have functional data available on a subset of patients and a possible next step for our research is a multi-modality study approach, for example using resting state functional MRI and DTI to describe whole brain differences in functional and structural connectivity and the relationship with specific symptom dimensions.

Advances in automated MRI tools have also recently included a hippocampal subfield segmentation package (Fischl 2012). At this point, manual tracing remains the gold standard (Wisse et al. 2014), however it is a labour intensive process, and automated segmentation is a useful way for us to look at hippocampal subfields and the hippocampal-amygdala complex that are important in both schizophrenia (Ho et al. 2017) and childhood trauma (Teicher et al. 2016).

Finally, I along with other members of our neuroimaging team, recently joined the ENIGMA (Enhancing Neuroimaging Genetics through Meta-analysis) consortium, a network that brings together researchers in imaging genetics for large collaborative neuroimaging projects (Thompson et al. 2014). One of the limitations of neuroimaging studies relate to small sample sizes and although inter-site discrepancies need to be considered, large collaborations that include low and middle-income countries are important.

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APPENDIX 1: SUPPLEMENTARY PUBLICATIONS

Additional publications first authored or co-authored by the PhD candidate that are related to the parent study cohort:

Asmal, L., Mall, S., Kritzinger, J., Chiliza, B., Emsley, R., & Swartz, L. (2011). Family therapy for schizophrenia: Cultural challenges and implementation barriers in the south african context. *African Journal of Psychiatry, 14*(5), 367-371.

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