Large-scale brain connectivity in schizophrenia in relation to auditory verbal hallucinations and familial liability

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Large-scale brain connectivity in schizophrenia in relation to auditory verbal hallucinations and familial liability

De rol van grootschalige hersenconnectiviteit in auditieve verbale hallucinaties en familiale kwetsbaarheid voor schizofrenie

(met een samenvatting in het Nederlands)

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General Introduction

1

Research into what we now understand as schizophrenia began in the mid-19th century, when neurologists and psychiatrists started to recognize psychosis as a disease entity, and possibly a result of brain abnormalities. Early pioneers such as Theodor Meynert and Carl Wernicke, pointed to abnormal association between brain regions as the root of what was at the time referred to as *insanity* (Beer, 1996). Since then, many prominent researchers made great efforts to expand our knowledge of the biological mechanism of schizophrenia (Tandon et al., 2009). In the past few decades, research of schizophrenia was greatly advanced by the implementation of *in vivo* neuroimaging techniques in clinical settings. Findings from early research and neuroimaging studies led to a popular hypothesis that schizophrenia results from a disruption of interactions between spatially distinct brain regions (Friston et al., 2016; Friston and Frith, 1995). The dysconnectivity hypothesis inspired many studies to examine large-scale brain functional interactions and underlying white matter pathways. Yet, many questions remain. A few of these questions are explored in this thesis: 1) are there abnormality of functional connectivity between the two hemispheres in patients; 2) if so, are such connectivity abnormalities pertinent to specific symptoms; 3) do patients show white matter connectivity changes examined by the free-water imaging technique; 4) are these identified functional and structural connectivity disruptions exist in unaffected siblings of patients who share familial risk of schizophrenia?

Studies presented in this thesis aim to address above questions. In the following paragraphs, I will first briefly present the clinical characteristics of schizophrenia, then introduce neuroimaging techniques to examine macroscopic brain functional and structural connectivity, followed by reviewing some connectivity studies of schizophrenia and finally, bring up the aim and outline of this thesis.

Schizophrenia

Schizophrenia, a debilitating and chronic neuropsychiatric disorder, affects around 0.7 ~ 1% of the population worldwide (Jablensky et al., 1992; McGrath et al., 2008). The disease typically strikes people in their early 20s and persists in about two thirds of affected individuals (Ferrari et al., 2012). Despite the prolonged illness course, patients with schizophrenia often suffer from pervasive loss of abilities, including cognitive deficits, interpersonal difficulties, and occupational impairments. In addition, patients have a two- to three-fold higher mortality rate than the general population (Saha et al., 2007). Schizophrenia is ranked among the top ten leading causes of disease-related disability (World Health Organization, 2001), and accounts for 7.4% of disease burden attributed to mental and substance use disorders (Whiteford et al., 2013).

Symptoms of schizophrenia

A diagnosis of schizophrenia encompasses a heterogeneous constellation of clinical signs and symptoms, including positive symptoms, negative symptoms, and cognitive deficits (American Psychiatric Association, 2000; Andreasen, 1995). Positive symptoms refer to false perceptions and beliefs, disorganized thought and speech, and catatonic symptoms. These symptoms tend to respond well to anti-psychotic medications and have been hypothesized to result from hyperdopaminergia in the striatal system (Howes and Kapur, 2009). Negative symptoms are characterized by diminished motivation, behavioral deficits and loss of emotional vibrancy, which may precede illness onset and persist after psychotic remission. Cognitive dysfunction involves impairment of attention, executive functions, verbal and working memory functions, and relates to poor functional outcomes in patients. Negative and cognitive symptoms do not respond well to current antipsychotic treatments, suggesting a different underlying neurochemical pathway from positive symptoms (Lewis and Lieberman, 2000).

Among positive symptoms, auditory verbal hallucinations (AVH), defined as “hearing voices” in the absence of actual auditory stimulation (Shergill et al., 2000), are commonly reported in schizophrenia, affecting between 40% to 80% of patients (Aleman and Larøi, 2008). AVH are a common form of hallucinations, and cause significant distress due to its uncontrolled nature and often negative content. AVH can also be experienced in patients with other neurological and psychiatric disorders such as Alzheimer’s disease (Bassiony and Lyketsos, 2003), Parkinson’s disease (Inzelberg et al., 1998), substance abuse (Perälä et al., 2010), affective disorder (Baethge et al., 2005), and even in the healthy population (Sommer et al., 2010). The neural mechanism of AVH has not been fully clarified, but several cognitive and neurological models have been proposed. These models suggest the involvement of both hyperactivity in speech perception regions and impairments in top-down monitoring processes (Allen et al., 2008; Jones, 2010; Laroi et al., 2012). AVH may be also related to a failure to inhibit memory intrusions, in association with a deactivation of the parahippocampal gyrus (Diederen et al., 2010). More research is still required to understand the neural mechanisms of AVH and to examine feasible assessment and treatment options.

Genetic basis for schizophrenia

Like many neuropsychiatric disorders, schizophrenia results from complex interactions among multiple genetic and environmental factors (Owen et al., 2016). Schizophrenia is a highly heritable disease, with an estimated heritability rate of 67 ~ 81% (Cardno and Gottesman, 2000; Hilker et al., 2018). As epidemiological studies show, concordance rates for schizophrenia are 41 ~ 65% in monozygotic twins and 0 ~ 28% in dizygotic twins (Cardno and Gottesman, 2000). The risk for schizophrenia is about ten times higher in first degree

relatives of patients as compared to individuals with no affected relatives (Lichtenstein et al., 2009; Mortensen et al., 1999). This genetic risk for schizophrenia is partially expressed as brain morphology alterations. For example, (poly)genetic variants have been associated with brain volume (Harrisberger et al., 2016; Terwisscha van Scheltinga et al., 2013) and cortical gyrification (Liu et al., 2016). As indicated in twin studies, shared genetic variations seem to exist between disease liability and structural brain connectivity (Bohlken et al., 2016). Meta-analytical evidence suggests that schizophrenia risk variants have a larger penetrance on brain structural and functional measurements than cognitive measurements (Rose and Donohoe, 2013). That being said, there are also negative results regarding the association between genetic risks of schizophrenia and brain imaging phenotypes (Franke et al., 2016; Voineskos et al., 2016), which may be partly related to different brain regions and measurements (cortical volume, thickness, gyrification, connectivity etc.) being investigated (Franke et al., 2016). Studies of discordant twins and non-psychotic siblings are informative to identify neuroimaging features as potential intermediate phenotypes to psychopathology.

Environmental risk factors

Genetic predisposition alone cannot explain the full psychopathology of schizophrenia, and should be considered in the context of environmental effects (van Os et al., 2010). Early environmental adversities such as maternal stress and prenatal exposure to infection and nutritional deficiencies are more prevalent in schizophrenia patients than controls (Brown and Derkits, 2010). Other factors, such as childhood trauma, cannabis use, socioeconomic status, and infections have also been related to increased risk for the illness (Brown, 2011; Linszen and van Amelsvoort, 2007). For example, cannabis consumption can lead to psychotic experiences (Linszen and van Amelsvoort, 2007), especially in individuals with a genetic risk for schizophrenia, suggesting an interaction between environmental factors and familial risk (Genetic Risk and Outcome in Psychosis (GROUP) Investigators, 2011). Factors related to socioeconomic status, such as living in an urban environment, and being an ethnic minority also confer greater risk (Brown, 2011).

Brain connectivity characterized with magnetic resonance imaging (MRI)

Since the 1980s, the application of *in vivo* neuroimaging techniques such as MRI has greatly expanded our understanding of the brain (Mansfield et al., 1978). The brain is an intricate, complex system, consisting of around 86 billion neurons and a roughly equal number of nonneuronal cells (Azevedo et al., 2009). On the macroscopic level, the brain can be divided into spatially distinct grey matter regions (mainly neuronal cell bodies) and their connections via white matter fiber bundles (densely packed myelinated axons). The different types of brain tissues can be discerned using different MRI scanning protocols. Functional magnetic

resonance imaging (fMRI) is able to characterize brain activity due to related changes in regional blood flow and blood oxygenation level (Ogawa et al., 1990). Diffusion-weighted magnetic resonance imaging (dMRI), based on the diffusion profile of water molecules, can indirectly reflect the coherence and direction of white matter fiber bundles.

The brain is a sophisticatedly connected system. In MRI studies, functional connectivity is defined as the statistical dependency of blood oxygen level dependent (BOLD) fluctuations between brain regions (Fox and Raichle, 2007; Friston et al., 1993) (Figure 1). Structural connectivity commonly refers to white matter fiber bundles that form the information transfer highways of the brain. The delicate coordination between distinct brain regions supports many aspects of behavior performances and cognitive abilities, such as intelligence (Song et al., 2008; van den Heuvel et al., 2009), reading ability (Beaulieu et al., 2005) and working memory (Hampson et al., 2006) etc. Impairments of functional and structural connectivity between brain regions have been linked to various neuropsychiatric conditions, including Alzheimer's disease, depression, schizophrenia, autism, attention deficit hyperactivity disorder, multiple sclerosis and epilepsy (Greicius, 2008).

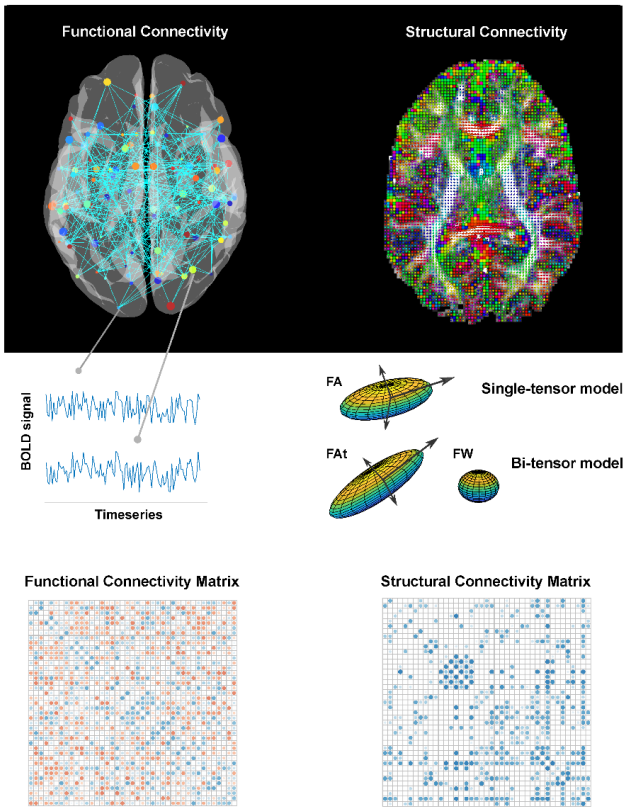

Figure 1 Brain functional and structural connectivity derived from MRI scans. *Left:* functional connectivity is calculated as Pearson's correlation between regional time-courses extracted from BOLD-fMRI images. *Right*: structural connectivity is reconstructed from dMRI scans based on water molecule diffusion. Different mathematical models (single-tensor models, bi-tensor models etc.) can be applied to fit the diffusion profile.

Resting-state functional connectivity

The fMRI technique was initially used to identify brain activation based on BOLD signal changes during a task or stimulus (Raichle and Mintun, 2006). Task-based fMRI studies provide many insights into the behavioral correlates of functionally specialized brain regions. More recently, researchers have been measuring brain activity during rest without specific tasks or goals using resting-state fMRI. Research on resting-state fMRI is motivated by two pieces of evidence: First, studies show that the brain is about 2% of the total body weight, but consumes 20% of its energy. Interestingly, most of the brain's oxygen consumption and

glucose utilization support ongoing neuronal activity during rest, while task-induced brain activity increases its metabolism by less than 5% (Fox and Raichle, 2007; Raichle and Mintun, 2006). Second, as Biswal et al., (1995) found, the co-activated sensorimotor cortex during hand movement also exhibited a high temporal coherence during rest. This allows resting-state functional connectivity to be used to capture functionally relevant brain networks. Patterns of these functional networks exhibit high spatial consistency across subjects (Damoiseaux et al., 2006), and can be identified in different levels of consciousness, such as falling asleep (Fukunaga et al., 2006) or anesthesia (Vincent et al., 2007). Thus, resting-state functional connectivity is believed to reflect trait characteristics of organizational patterns of the brain independent from task requirements.

Because resting-state fMRI study is easy to implement and its results are not influenced by task performance, the technique is particularly useful in research of neuropsychiatric diseases. It is commonly used in both model-based analyses and model-free (or data-driven) analyses (Lee et al., 2013; Li et al., 2009; van den Heuvel and Hulshoff Pol, 2010). Model-based approaches such as regions of interest (ROI) analyses require an *a priori* choice of brain regions, selected based on prior neuroanatomical knowledge, or areas implicated by previous studies. Model-free methods encompass a set of techniques to decompose data, extract features, and classify or group data into clusters without any prior assumptions. Widely applied model-free methods include principle component analysis (PCA) (Friston et al., 1993), independent component analysis (ICA) (Beckmann et al., 2005; Calhoun et al., 2001), hierarchical clustering analysis (Cordes et al., 2002; van den Heuvel et al., 2008), and multivariate pattern classifications (Pereira et al., 2009). Both model-based and model-free methods have strengths and disadvantages, with the methodological choice always dependent on the questions to be addressed (Li et al., 2009). Studies in this thesis will adopt a model-based approach to investigate functional connectivity between previously implicated regions of interest involved in AVH (**Chapter 2**). **Chapter 3 and 4** focus on functional interhemispheric connectivity in relation to AVH symptoms and genetic predisposition to schizophrenia. The advantage of model-based ROI analysis is its ability to integrate prior knowledge and to avoid testing irrelevant effects. Moreover, the interpretation of its results is more straightforward than for model-free methods. However, it is biased by the assumptions of researchers, and is not suitable to deal with high dimensional data.

White matter structural connectivity

The dMRI technique provides information on white matter microstructural characteristics through water molecule diffusion (i.e. Brownian motion) in fiber bundles. Water diffusion in white matter fiber bundles (consisting of axons running in parallel) is restricted by boundaries of axonal membrane and myelin sheath, and is thus largely parallel to the direction of axons

(Le Bihan et al., 2001). One particular dMRI application, diffusion tensor imaging (DTI), models water displacement using a diffusion tensor model (Pierpaoli et al., 1996; Tournier et al., 2011). The fitted tensor is mathematically described as a 3 x 3 matrix, with three orthogonal eigenvectors representing the diffusion direction, and associated eigenvalues to quantify the diffusion magnitude. From the diffusion tensor, a set of DTI measurements can be calculated, including the fractional anisotropy (FA) (Basser and Pierpaoli, 1996). FA is a scalar index ranging from 0 (i.e. water diffusion is isotropic) to 1 (i.e. water diffusion is anisotropic), and is regarded as a proxy for white matter fiber integrity. The DTI technique and its derived measurements have been widely applied to study white matter fiber integrity in healthy populations (Johansen-Berg and Rushworth, 2009; Pierpaoli et al., 1996) and in neuropsychiatric disorders (Horsfield and Jones, 2002; Rowland et al., 2009).

Although FA is proven to be a sensitive measurement to fiber integrity changes with diseases (Horsfield and Jones, 2002) and aging (Westlye et al., 2010), challenges still remain regarding the biological mechanism underlying FA changes (Jones, 2010). For example, in fibers adjacent to ventricles, cerebral atrophy, or neuroinflammation, the diffusion signal of brain tissue can be contaminated by the diffusion signal of (adjacent) free water resulting in inaccurate fiber modelling (Alexander et al., 2001; Pierpaoli and Jones, 2004). This issue led Pasternak et al., (2009) to develop a bi-tensor modelling method to separately model the free water component and the diffusion profile in brain tissue. This free-water imaging technique produce two measurements: a free-water fraction (FW) which is sensitive to free-water accumulation possibly due to neuroinflammation or neurodegeneration, and a fractional anisotropy in tissue compartment (FA_t) as an indicator of fiber integrity. The free-water imaging technique has been reported to improve specificity of fiber modelling (Bergamino et al., 2017) and exhibits better test-retest reproducibility than the conventional tensor modelling technique (Albi et al., 2017). Moreover, FW itself can provide valuable information as it may be linked to neuroinflammation and higher FW has been reported in the acute phase of schizophrenia (Lyall et al., 2018; Pasternak et al., 2012).

Dysconnectivity hypothesis of schizophrenia

Advances of neuroimaging techniques provide an opportunity to examine large-scale cortical-cortical connectivity in schizophrenia. The modern version of the “dysconnectivity hypothesis” was explicitly formulated by Friston and Frith (1995), “…*schizophrenic phenomena are best understood in terms of abnormal interactions between different areas*…”. Several models have been postulated under the dysconnectivity hypothesis. Stephan et al., (2009) and Friston et al., (2016) suggested that the core pathology of schizophrenia is abnormal N-methyl-D-aspartate receptor (NMDAR) mediated synaptic plasticity, possibly due to modulation of other neurotransmitters. Crow et al., (1997)

suggested that impaired inter-hemispheric connectivity possibly disrupts the establishment of normal cerebral asymmetry and thereby gives rise to schizophrenic symptoms. Andreasen et al., (1998) suggested that dysconnectivity in prefrontal-thalamus-cerebellum circuitry would disrupt patients' cognitive functions and account for a broad range of clinical symptoms. These hypotheses are not exclusive to each other, and they may reflect the heterogeneous pathological pathways to schizophrenia.

In accordance with the dysconnectivity hypothesis, previous studies suggested that the emergence of AVH not only involves abnormal activation in focal brain regions, but also relates to interactions among cortical and subcortical areas (Alderson-Day et al., 2015; Northoff and Qin, 2011). Compared to healthy controls or schizophrenia patients without hallucinations, AVH patients have impaired functional and structural connectivity among speech processing regions (i.e. the left superior temporal gyrus and nearby regions, and Broca's area) (Hubl et al., 2004), and reduced inter-hemispheric connectivity between these areas and their right hemisphere homologues (Ćurčić-Blake et al., 2015; Gavrilescu et al., 2010), and connectivity between speech processing areas and other cortical regions such as the anterior cingulate cortex, insula, temporo-parietal cortices, and (para)hippocampus (Clos et al., 2014; Vercammen et al., 2010). However, as most previous studies recruited chronic patients under antipsychotic treatment, it is unclear how prolonged illness and various medications influence prior results, and whether the identified neural abnormalities represent primary pathology or secondary effects after initial insults.

Schizophrenia patients have also been suggested to exhibit impairments in connectivity between corresponding regions in the two hemispheres, i.e. (homotopic) interhemispheric connectivity. Homotopic brain regions are directly connected by association fibers and exhibit a high level of synchronization (Stark et al., 2008; Zuo et al., 2010). As an important aspect of hemispheric cooperation, interhemispheric connectivity may facilitate higher order cognitive and emotional functions (Gazzaniga, 2000), and reduced connectivity in schizophrenia patients may result in difficulty to integrate bilaterally presented information (Mohr et al., 2008, 2000). Previous studies have reported lower interhemispheric connectivity in patients in several regions: the pre-, post-central gyrus, superior temporal gyrus, precuneus, occipital gyrus and cerebellum (Guo et al., 2014; Hoptman et al., 2012; Li et al., 2015). Yet, whether such abnormalities are results of familial risk for schizophrenia or illness manifestation effects has not been examined. It is also unclear whether abnormalities in interhemispheric connectivity are related to hemispheric specialization characterized by intrinsic connectivity (Mueller et al., 2015; Wang et al., 2014).

White matter connectivity examined with the dMRI technique has been shown to be impaired in schizophrenia, which is supported by postmortem (Glausier and Lewis, 2013) and genetic studies (Osimo et al., 2018). Previous studies showed that schizophrenia

patients are characterized by synaptic loss (Glausier and Lewis, 2013; Osimo et al., 2018), and lower levels of myelination and reduced oligodendroglia cells (Davis et al., 2003; Flynn et al., 2003; Uranova et al., 2004), which may contribute to lower fiber integrity reflected by FA and other diffusion measurements. Previous studies have reported widespread white matter abnormalities in schizophrenia, including frontotemporal connections, corpus callosum, cingulate bundle, and subcortical-cortical connectivity (for reviews and meta-analyses refer to: Ellison-Wright and Bullmore, 2009; Kelly et al., 2018; Kubicki et al., 2007; Melonakos et al., 2011). Some studies have reported lower FA values in patients' non-psychotic siblings as compared to healthy controls (Camchong et al., 2009; Skudlarski et al., 2013), while others did not find significant group-differences (Boos et al., 2013; Harms et al., 2015). These inconsistent results may partly be due to the non-specific nature of FA to underlying biological aberrances such as demyelination, axon impairment, and extracellular water accumulation. The latter issue can be partly corrected by the newly developed free-water imaging technique (Pasternak et al., 2009).

Aim of this thesis

The research in this thesis aims to assess functional and structural connectivity in schizophrenia using MRI techniques, and to contribute new evidence to elucidate connectivity abnormalities in schizophrenia in relation to symptomology and familial risk for the disorder.

Outline by chapters

Research presented in this thesis examines schizophrenia-related functional connectivity and white matter structural connectivity using different MRI techniques. In **chapter 2**, we will focus on resting-state functional connectivity in first-episode, medication-naïve patients with auditory verbal hallucinations (AVH) versus patients without hallucinations (non-AVH) and healthy controls. Based on previous literature, we selected eighteen brain regions of interest, previously implicated in hallucinatory experiences. Functional connectivity among these regions will be compared across the three subject groups. **Chapter 3** is based on the same dataset as **chapter 2**, but focuses on inter-hemispheric functional connectivity, to examine whether patients with and without AVH show impairments in the coordination between the two hemispheres. In **chapter 4**, we further examine inter-hemispheric connectivity in relation to hemispheric specialization in schizophrenia patients, their unaffected siblings and healthy controls. **Chapter 5** explores white matter structural connectivity integrity in patients, siblings and controls, using the free-water imaging technique. This technique enables us to examine white matter integrity as well as the existence of free-water accumulation in patients, which

may indicative of neurodegenerative or neuroinflammatory processes, respectively. The inclusion of unaffected siblings of schizophrenia patients in **chapters 4** and **5** provides an opportunity to discover whether potential functional and structural dysconnectivity can be attributed to familial risk for schizophrenia. Finally, **chapter 6** summarizes the findings presented in this thesis, and discusses methodological considerations and implications for future research.

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Resting-state functional connectivity in medication-naïve schizophrenia patients with and without auditory verbal hallucinations: a preliminary report

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Abstract

Auditory verbal hallucinations (AVH) are a cardinal feature of schizophrenia that has been associated with activation in language processing areas, in concert with higher-order cognitive brain networks. It remains to be determined whether, and if so how, the functional dynamics between these brain regions contributes to the emergence of AVH. The current study recruited 36 first-episode medication-naïve schizophrenia patients, including 18 patients with AVH, 18 patients free of AVH and 18 controls matched on age, gender and level of education. Resting-state functional MRI images were acquired for every subject and used to map functional brain connectivity. We compared functional connectivity in 18 bilateral regions of interest implicated by previous AVH studies among the three subject groups, with the aim of detecting patterns of dysconnectivity unique to or most pronounced in AVH patients. Results showed that AVH patients are characterized by dysconnectivity in neural circuitry involving the anterior cingulate cortex, insular cortex and language-related regions, comparing with both controls and non-AVH patients. Current findings suggest that abnormality in speech-sensitive areas and their functional cooperation with cortical regions involving in source monitoring and salience detection functions may contribute to the occurrence of AVH.

Keywords

schizophrenia; auditory verbal hallucinations; functional connectivity; speech monitoring

Introduction

Auditory verbal hallucinations (AVH), or 'hearing voices', are the perception of speech in the absence of an external auditory stimulus. AVH are common to schizophrenia, affecting between 40% to 80% of patients (Aleman and Larøi, 2008), but also occur in various other clinical disorders (Larøi, 2012) and even in a subpopulation of healthy individuals (Sommer et al., 2010).

Investigating the neural mechanism of AVH with magnetic resonance imaging (MRI) has been gaining in popularity in recent years. Meta-analyses of functional MRI (fMRI) and positron-emission tomography (PET) studies suggested that auditory hallucinations were associated with abnormal focal activations in language related areas (e.g., inferior frontal gyrus, superior temporal gyrus and nearby cortical areas) and other regions of the cortex (Jardri et al., 2011; Kompus et al., 2011; Kühn and Gallinat, 2010). Abnormal functional connectivity among these brain regions has also been proposed to contribute to hallucinatory experiences (Clos et al., 2014; Hoffman and Hampson, 2012; Sommer et al., 2012; Vercammen et al., 2010). For example, studies have suggested that AVH may result from hyperactivity of the auditory cortex (bottom-up) in combination with abnormal (top-down) modulation by higher-order cognitive brain regions within the default-mode network and salience network (Alderson-Day et al., 2015; Allen et al., 2008; Northoff and Qin, 2011). However, challenges remain in functional connectivity studies of AVH, including the prevalent use of antipsychotic medications in schizophrenia patients, which has been suggested to alter resting-state functional connectivity (Bolding et al., 2012; Lui et al., 2010; Sambataro et al., 2010). Moreover, as direct comparisons of patients with versus those without AVH are scarce, it is difficult to determine whether the observed abnormalities in functional connectivity are AVH-specific, or general aspects of schizophrenia.

In the current study, we examined patterns of dysconnectivity related to AVH among regions consistently implicated by previous AVH studies, including the superior and middle temporal gyrus, inferior parietal lobule, inferior frontal cortex (pars triangularis and pars opercularis), anterior cingulate cortex (caudal and rostral part), postcentral gyrus and insula. To examine functional dysconnectivity in the absence of the potential confounds of psychotropic medications, we acquired resting-state fMRI data in first-episode medication-naïve schizophrenia patients and a group of demographically matched healthy controls. Comparing patients with versus those without AVH and healthy controls, we aim to identify functional dysconnectivity unique to, or most pronounced in, patients with AVH.

Methods

Subjects

A total of 54 subjects participated in this study, including 36 first-episode medication-naïve schizophrenia patients – 18 with auditory verbal hallucinations (AVH group) and 18 without hallucinations (non-AVH group) – and 18 healthy controls (CON group). These subjects were selected from a larger sample of 120 first-episode schizophrenia patients from a National Basic Research Program of China (No. 2011CB707805). The majority of patients had taken anti-psychotic medications at the time of recruitment, but only unmediated patients were selected for this current study, as described previously in a study on inter-hemispheric connectivity (Chang et al., 2015). The study was approved by the ethics committee of the Xijing Hospital, affiliated with the Fourth Military Medical University, Xi'an, China. All participants gave their written informed consent.

Patients were recruited from the outpatient clinic of the psychiatry department, meeting a diagnosis of schizophrenia through consensus of two senior clinical psychiatrists using the Structural Clinical Interview for DSM-IV (SCID) (First et al., 1995). Symptom severity was assessed using the Positive and Negative Syndrome Scale (Kay et al., 1987). Patients scoring 3 or more on PANSS hallucination item P3 were further evaluated for hallucination content and frequency. Those experiencing AVH at least once a day in the past four weeks were recruited in the AVH group. The non-AVH group included patients who scored 1 on P3 (absence of symptom) and did not experience hallucinations in the two years before recruitment. Healthy volunteers were recruited via advertisements and screened for the absence of DSM-IV axis I and II disorders. Exclusion criteria for all participants included a history of a neurological or severe medical disorder, substance abuse or dependency, prior electroconvulsive therapy or head injury resulting in loss of consciousness. All included participants were right-handed, matched on age, gender and education level (Table 1).

Table 1 Demographic and clinical characteristics of patients with AVH (*n* = 18), patients without AVH (*n* = 18) and healthy controls (*n* = 18)

Characteristics	AVH Patients	non-AVH Patients	Controls	*p* value
Age	22.56 ± 6.73	22.67 ± 3.80	24.44 ± 3.73	0.46
Sex (male/female)	10 / 8	9 / 9	10 / 8	0.93
Education (years)	12.44 ± 2.31	12.56 ± 2.20	13.89 ± 3.50	0.22
Duration of illness (months)[a] median (IQR)	5 (1.5, 6)	4.5 (2.25, 12)	—	0.54
PANSS Total [b]	106.44 ± 13.55	88.06 ± 23.90	—	0.01
PANSS Positive [b]	31.11 ± 6.92	18.61 ± 8.68	—	0.00
PANSS Negative	25.78 ± 3.87	22.06 ± 10.43	—	0.17
PANSS General Psychopathology	49.56 ± 9.01	47.39 ± 9.84	—	0.49

[a] Duration of illness was determined from first meeting schizophrenia diagnoses until recruitment into this study. Due to non-normal distribution of illness duration, median and interquartile range (IQR) were reported instead of mean and standard deviation. Group comparison was performed using Kruskal-Wallis H test.

[b] Patients with AVH have significantly higher Positive and Negative Syndrome Scale (PANSS) total and positive symptoms than patients without AVH. Groups were matched for age, gender, and education level.

Image acquisition

Resting-state fMRI images and structural T1 scans were acquired for every subject on a 3.0-T Siemens Magnetom Trio Tim scanner. During image acquisition, the participants were instructed to lie still and keep their eyes closed without falling asleep. Subjects were judged to be awake at the start and end of scanning. Earplugs and a MRI-compatible head coil were used to minimize head motion and attenuate scanner noise.

Resting-state functional scans were collected using a gradient-echo echo-planar imaging (EPI) sequence (TR = 2000 ms, TE = 30 ms, flip angle = 90°, FOV = 220 × 220 mm^2). Whole-brain volumes comprised 33 contiguous transverse slices with 4 mm thickness, 0.6 mm gap and 3.40 × 3.40 mm^2 in-plane resolution. For every subject, 240 whole-brain volumes were acquired. T1-weighted high-resolution structural images were obtained using a three-dimensional magnetization prepared rapid acquisition gradient echo (3D MPRAGE) sequence with the following parameters: TR = 2530 ms, TE = 3.5 ms, flip angle = 7°, FOV = 256 × 256 mm^2, slice thickness = 1 mm, 192 slices with 0.5 mm gap.

Image Preprocessing

Data preprocessing was performed using the SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm/). Resting-state time-series were realigned to the first volume and then co-registered with the T1 image. Nuisance correction, including six realignment parameters, CSF and white matter signals was performed by linear regression. To further minimize the influence of head motion on resting-state fMRI data, each slice with a framewise displacement exceeding 0.5 mm – indicating excessive movement – was scrubbed following the procedure of Power et al. (2012), with framewise displacement defined as the sum of the absolute values of the derivatives of the six realignment parameters (Power et al., 2012; Van den Heuvel et al., 2013). There were no significant group-differences in either the number of deleted scans ($F = 1.52$, $p = 0.23$), or motion parameters after scrubbing ($F = 0.60$, $p = 0.56$). More detailed information is provided in the Supplementary Table 1. Time-series were bandpass filtered (0.01 – 0.05 Hz) to eliminate low frequency noise and influences of frequencies reflecting possible cardiac or respiratory oscillations.

Functional brain network reconstruction

Automated parcellation of the cortex into coherent regions was performed using Freesurfer's Desikan-Killiany atlas (Desikan et al., 2006), resulting in 82 brain areas for every subject. Functional connectivity between each pair of brain regions was defined as Pearson's correlation coefficient of regional time-series after Fisher's *r*-to-*z* transformation. Both negative and positive correlation values (i.e. the full connectivity matrix) were included and constituted a functional network for every subject.

Regions of interest

As implicated by previous research, the emergence of AVH has been related to abnormalities in brain regions underlying language processing and higher order functions (Allen et al., 2012). To investigate the association between functional interaction among

these regions and AVH occurrence in schizophrenia patients, we selected regions of interest (ROIs) that were reported at least twice among three recent meta-analyses of AVH-related brain activation (Jardri et al., 2011; Kompus et al., 2011; Kühn and Gallinat, 2010). The three meta-analyses included functional imaging studies (fMRI, PET) of schizophrenia spectrum patients with AVH using a quantitative meta-analytic approach. Two kinds of study design were included: 'state' studies examining hallucination-related functional activations versus resting-state in AVH patients, and 'trait' studies comparing functional activation during auditory tasks between patients and healthy or non-AVH psychotic controls. As a result, 18 bilateral regions (9 per hemisphere) were defined as ROIs (Figure 1 and Table 2) including the (1) superior temporal gyrus and (2) middle temporal gyrus which are involved in speech perception and comprehension (Hickok and Poeppel, 2007) and extend into the (3) inferior parietal lobule where the angular gyrus and supramarginal gyrus are located (Torrey, 2007). In addition, the (4) pars triangularis and (5) pars opercularis, which are part of the inferior frontal gyrus and comprise Broca's area in the left hemisphere (Poldrack et al., 1999), and (6) caudal and (7) rostral part of anterior cingulate cortex, which participate in cognitive monitoring (Botvinick et al., 2004; Carter et al., 1998) were included as ROIs. Finally, the (8) postcentral gyrus (McGuire et al., 1995) and (9) insula (Diederen et al., 2012; Sommer et al., 2008) were also reported by the majority of the AVH meta-analyses, and thus included as predefined ROIs. Resting-state functional connectivity of ROIs was examined for its relevance to AVH, i.e. connectivity that is altered exclusively, or most severely, in AVH patients.

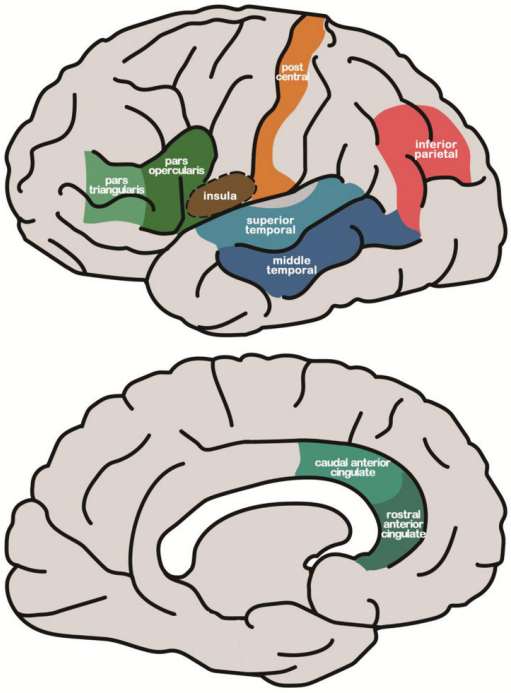

Figure 1 Lateral and medial view of predefined regions of interest (ROIs). ROIs were selected based on three recent meta-analyses (Jardri et al., 2011; Kompus et al., 2011; Kühn and Gallinat, 2010), as those cortical regions were implicated in AVH by at least two out of the three meta-analyses.

Table 2 Meta-analytical evidence on brain regions involved in AVH

Meta-analyses	Kühn and Gallinat	Jardri et al.	Kompus et al.
Characteristics of included studies			
Imaging Modality	PET, fMRI	PET, fMRI	PET, fMRI
Number of studies (participants)[a]	State:10 (85 AVH) Trait: 8 (81 AVH, 39 non-AVH, 69 CON)	10 (68 AVH)	State: 12 (103 AVH) Trait: 11 (204 AVH, 170 CON)
Study Design	Hallucination(state) Auditory task (trait)	Hallucination	Hallucination(state) Auditory task (trait)
Significance/Threshold	FDR $q < 0.01$, cluster threshold = 100 mm^3	FDR $q < 0.05$, cluster threshold = 200 mm^3	FDR $q < 0.05$, cluster threshold = 200 mm^3
Significant regions and peak Talairach coordinates			
Superior temporal gyri	(-44, -22, 0)	(-54, -44, 16)	(-52, -22, 16) (-54, -8, 0) (58, -44, 14)
Middle temporal gyri	(-56, -30, 0)		(54, -32, -4)
Inferior parietal lobule	(-55, -19, 16)	(-52, -20, 15)	(32, -40, 48)
Inferior frontal gyrus	(-48, 2, 6)	(-48, 10, 7) (42, 12, -10)	(40, 12, 16)
Anterior cingulate	(-9, 4, 37) (-42, 2,18) (-4, 26, 31)		(-10, 0, 40)
Postcentral gyrus	(-49, -17, 41) (36, -32, 50)		(-50, -24, 40)
Anterior insula		(-42, 0, 6) (44, 6, -4)	(-44, -2, 6)

[a] AVH: schizophrenia or schizophrenia spectrum patients with auditory verbal hallucinations; non-AVH: schizophrenia or schizophrenia spectrum patients without auditory verbal hallucinations; CON: healthy controls

Dysconnectivity pattern analysis

AVH-specific abnormalities

AVH-specific abnormalities were defined as those connections meeting two criteria: 1) showing a group-difference ($p < 0.05$, uncorrected) between AVH and CON groups; *and* 2) showing a group-difference ($p < 0.05$, uncorrected) between AVH and non-AVH patients, with a joint probability of detecting such an effect by chance of $p = 0.05 \times 0.05 = 0.0025$. Thereby, we set out to identify those connections associated with AVH in particular, rather than generally affected in schizophrenia. As exploratory findings, group-differences in connectivity with $p < 0.05$ in either AVH vs. CON or AVH vs. non-AVH comparisons were reported in Supplementary Table 2.

Step-wise effects

Next, we examined the presence of step-wise dysconnectivity effects, in which connectivity alterations (relative to controls) were most pronounced in the AVH group, while intermediate in the non-AVH patients. Step-wise effects were examined by Jonckheere-Terpstra analysis, which is a nonparametric test to detect ordered differences among three or more groups (Bewick et al., 2004; Collin et al., 2014). The *a priori* assumption tested with this analysis being AVH > non-AVH > CON. The Jonckheere–Terpstra statistic, indicating the extent to which connectivity strength in three groups follows the a priori assumptions, was calculated for each connection, and then compared with standard normal distribution to decide on statistical significance ($q < 0.05$, FDR corrected for multiple comparisons). An exploratory analysis was performed to test possible step-wise effects in the opposite direction, i.e., AVH < non-AVH < CON.

Validation of ROIs selection

The predefined ROIs examined in this study were selected based on majority vote among three meta-analyses of brain activation during AVH (Figure 1 and Table 2). To assess whether the observed results are specific to the selected ROIs, or possibly a reflection of whole brain connectivity deficits, a post-hoc analysis was performed. Here, for 10,000 permutations, 18 bilateral regions outside the predefined ROIs were randomly selected. Using these regions, the dysconnectivity pattern analysis was re-performed, testing for 'AVH-specific' and 'step-wise' effects among the randomly selected regions. The 10,000 iterations produced two null-distributions of connectivity patterns (Supplementary Figure 1). The number of significant findings in the main analyses were compared to the generated null-distributions and *p*-values were assigned as the proportion of iterations resulting in an equal or greater number of AVH-specific and step-wise effects.

Results

Dysconnectivity pattern analysis

AVH-specific abnormalities

As shown in Figure 2A, AVH-specific effects (uncorrected $p < 0.05$ in both AVH vs. CON and AVH vs. non-AVH comparisons) were observed in connections among the right caudal anterior cingulate and right insular cortex (AVH vs. CON: $p = 0.035$ | AVH vs. non-AVH: $p = 0.020$), right caudal anterior cingulate and left superior temporal gyrus (AVH vs. CON: $p = 0.049$ | AVH vs. non-AVH: $p = 0.014$), between the right insular cortex and left superior temporal cortex (AVH vs. CON: $p = 0.014$ | AVH vs. non-AVH: $p = 0.043$), and between the right insular cortex and left inferior parietal lobule (AVH vs. CON: $p = 0.024$ | AVH vs. non-AVH: $p = 0.049$). Of note, a post-hoc analysis comparing connectivity strength of these identified AVH-specific connections found no significant differences between non-AVH schizophrenia patients and controls (all $p > 0.05$), underscoring that these connections do not appear to be affected in schizophrenia in general, but to be associated with AVH more specifically.

Step-wise effects

Jonckheere-Terpstra analysis yielded five connections showing a significant step-wise pattern of functional connectivity differences, with AVH patients showing the most pronounced increase in functional connectivity relative to controls, and intermediate levels in non-AVH patients (Figure 2B). The connections were between the left pars opercularis and bilateral postcentral gyrus, between the right insular cortex and left superior temporal gyrus, right insular cortex and left inferior parietal lobule, and also between the right caudal anterior cingulate cortex and right postcentral gyrus (all $p \leq 0.002$, surviving FDR corrected $q < 0.05$). No significant results were detected examining AVH < non-AVH < CON.

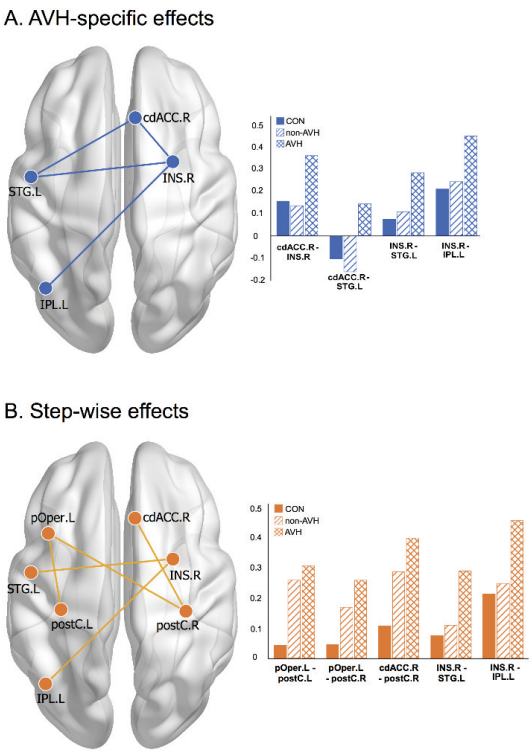

Figure 2 (A) AVH-specific effects detected by overlap of group differences between AVH vs. CON and AVH vs. non-AVH comparisons. The blue lines on the left side indicate altered connectivity in AVH patients compared to both CON and non-AVH patients (with a joint probability equal to $p = 0.05 \times 0.05 = 0.0025$). Group mean of connectivity strength is depicted on the right. (B) Step-wise dysconnectivity detected by Jonckheere–Terpstra test. Compared to controls, patients with AVH exhibited more pronounced changes than non-AVH patients ($q < 0.05$, FDR corrected), as reflected from the bar chart of corresponding connectivity group mean on the right side.
Abbreviations: L – left hemisphere; R – right hemisphere; STG - superior temporal gyrus; IPL - inferior parietal lobule; pOper – pars opercularis; cdACC – caudal anterior cingulate cortex; postC – postcentral gyrus; INS – insula

Validation of ROIs selection

To assess the validity of the ROI selection, a permutation analysis was performed in which brain regions outside the predefined ROIs were included at random (Supplementary Figure 1). The random selection procedure was repeated 10,000 times to generate two empirical distributions of AVH-specific and step-wise effects among any set of 18 randomly selected

brain areas. This analysis indicated that the predefined ROIs produced significantly more AVH-specific (p = 0.004) and step-wise (p = 0.033) effects than randomly selected regions, verifying that our main results were not driven by global connectivity effects in patients.

Discussion

We examined resting-state functional connectivity patterns among AVH-related brain regions in a group of first-episode medication-naïve schizophrenia patients with AVH, versus first-episode medication-naïve schizophrenia patients without hallucinations. Compared to both healthy controls and non-AVH patients, AVH patients showed unique functional dysconnectivity effects in a neural circuit involving the anterior cingulate cortex, insular cortex and language-related areas (superior temporal gyrus, inferior parietal lobule). Taken together, our study suggests that functional dysconnectivity exists in first episode schizophrenia patients free from antipsychotic treatment, and – perhaps more importantly – that patients with AVH show a unique dysconnectivity pattern that may differentiate them from schizophrenia patients without AVH symptoms.

The observed functional dysconnectivity among the anterior cingulate, insular cortex and language related areas in AVH patients is consistent with findings of previous AVH studies. Altered brain activation in the anterior cingulate cortex has been reported to precede (Hoffman et al., 2008) and accompany the occurrence of hallucinations (Lennox et al., 2000; McGuire et al., 1993; Shergill et al., 2000). The potential contribution of this region to the occurrence of hallucinations has been attributed to its proposed monitoring function (Carter et al., 2001, 1998) and its modulatory effects on speech-sensitive auditory regions (Hunter et al., 2006). In addition, the anterior cingulate cortex shares dense connectivity with the insular cortex on both the structural and the functional level (Menon and Uddin, 2010; Van den Heuvel and Sporns, 2013). Co-activation of these regions has been associated with the detection and response to salient stimuli in the environment (Dosenbach et al., 2007, 2006; Seeley et al., 2007). Our current findings are consistent with the hypothesis that AVH arise from abnormalities in the intrinsic activity of the auditory cortex in association with cortical areas involving in monitoring and salience detection (Allen et al., 2008; Palaniyappan and Liddle, 2012; Woodward and Menon, 2013). Although the AVH-specific dysconnectivity was only identified in the right anterior cingulate and insular cortex, we did see a trend-level of abnormality between the left anterior cingulate and the right insular (CON vs. AVH: p = 0.064 | AVH vs. non-AVH: p = 0.067), suggesting that there may be similar effects in the left hemisphere that we did not pick up due to our modest sample size. Based on previous evidence and current results, we argue that aberrant functional cooperation of speech-sensitive brain regions with the anterior cingulate and insular cortex is likely to be an AVH

specific aberrance, which may cause AVH patients to inaccurately attach salience to self-generated voices and misinterpret them as external stimuli.

Connectivity between the left pars opercularis and bilateral postcentral gyrus as well as between right postcentral gyrus and anterior cingulate cortex showed more pronounced alterations in the AVH patients than non-AVH patients. The left pars opercularis is part of Broca's area, which engages in phonologic and semantic processing (Poldrack et al., 1999). The postcentral gyrus is the location of the primary somatosensory cortex and has been suspected to involve in integrating information about mouth movements into the process of speech perception (Skipper et al., 2005). Such language-related abilities may be disrupted in all schizophrenia patients (DeLisi, 2001), but most prominently altered in patients with AVH.

We note that all AVH-related dysconnectivity found in the current study identified increased connectivity strength in the AVH patients, in line with previous studies of AVH reporting enhanced functional connectivity (van Lutterveld et al., 2014). Functional studies have found that auditory hallucinations are often associated with increased activity in temporal regions (Dierks et al., 1999; Shergill et al., 2000). In addition, enhanced activation in speech-sensitive regions has been reported to accompany activations in anterior cingulate cortex (Hunter et al., 2006; Szechtman et al., 1998). Therefore, the occurrence of AVH could, at least partly, result from increased interaction between speech processing areas and anterior cingulate cortex. Other explanations of increased functional connectivity include compensatory mechanism of brain functions, i.e. patients might engage more regions than controls to compensate focal abnormalities (Skudlarski et al., 2010; Tan et al., 2007) and re-organization of crucial (hub-) regions of the cortex (Fornito and Bullmore, 2014; Van den Heuvel and Fornito, 2014).

Some points need to be taken into account when interpreting our findings. First, in the current study, we compared functional connectivity between patients with versus those without AVH, aiming to distinguish changes in functional connectivity unique to auditory hallucinations. Considering that the majority of schizophrenia patients experience AVH (Aleman and Larøi, 2008), the non-AVH patients might be argued to form a biologically distinct subgroup of schizophrenia patients. However, we did not find any significant differences between the patient groups in demographic characteristics or PANSS negative and general psychopathology scores (Table 1). Second, the AVH and non-AVH patients differ significantly in PANSS positive and total scores. To ensure that our findings were not merely driven by group-differences in overall symptom severity, we conducted a post-hoc analysis in which the sum of PANSS negative and general psychopathology was taken as covariate in comparing connectivity strength between the two patient groups. Results showed that group-differences of the four identified AVH-specific dysconnectivity were still significant or marginally significant ($p \leq 0.09$), suggesting that the reported connectivity

differences were mainly related to group-differences in positive symptoms, rather than the AVH-group being generally more ill. Third, the whole scanning session lasted around fifteen minutes, during which time subjects may have fallen asleep or experienced hallucinations (Tagliazucchi and Laufs, 2014). Following common practice, we examined wakefulness of every subject before and immediately after scanning, and kept scanning duration short. Nonetheless, we cannot rule out this possibility due to the uncontrollable nature of resting-state design, and the interpretation of our results should therefore also consider evidence from other imaging modalities. Last, our study is limited by the relatively small sample size. Yet, an important strength of the current study is the recruitment of a sample of first-episode medication-naïve patients. All previous studies assessing connectivity in patients with and without AVH were confounded by medication effects. This study only includes medication free patients, which makes it possible to disentangle disease processes from medication effects.

In conclusion, our study examined functional connectivity among cortical brain regions implicated in AVH by previous meta-analyses in a group of first-episode medication-naïve patients with versus those without auditory hallucinations. Our results show that AVH patients exhibit stronger functional connectivity between the anterior cingulate cortex, insular cortex and language-related regions than non-AVH patients and healthy controls. These findings point to a neural circuitry dysfunction – involving speech processing regions, self-monitoring and salience detection systems – to be particularly relevant to the occurrence of hallucinations in schizophrenia. Furthermore, we show that such deficits are already established in the early stage of the illness and are independent of the effects of antipsychotic medication.

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Supplementary material of

Resting-state functional connectivity in medication-naïve schizophrenia patients with and without auditory verbal hallucinations: a preliminary report

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Supplementary Table 1 Head motion parameters and group-comparisons

Supplementary Table 2 Connectivity differed between the AVH patients and the other two groups

Supplementary Table 3 Jonckheere-Terpstra statistic on step-wise dysconnectivity

Supplementary Figure 1 Post-hoc analysis testing the specificity of selected ROIs

Supplementary Table 1 Head motion parameters and group-comparisons

	CON	non-AVH	AVH	ANOVA	
				F	*p*
FD* before scrubbing mean (std)	0.20(0.06)	0.22 (0.09)	0.23 (0.12)	0.65	0.53
Number of deleted scans	6.89 (10.74)	13.50 (20.15)	19.89 (31.33)	1.52	0.23
FD after scrubbing mean (std)	0.18 (0.05)	0.19 (0.05)	0.17 (0.06)	0.60	0.56

*FD: framewise displacement was defined as the sum of the absolute values of the derivatives of the three translational parameters and three rotational parameters. Each slice with a FD value exceeding 0.5 mm – indicating excessive movement – was scrubbed.

Supplementary Table 2 Connectivity differed between the AVH patients and the other two groups

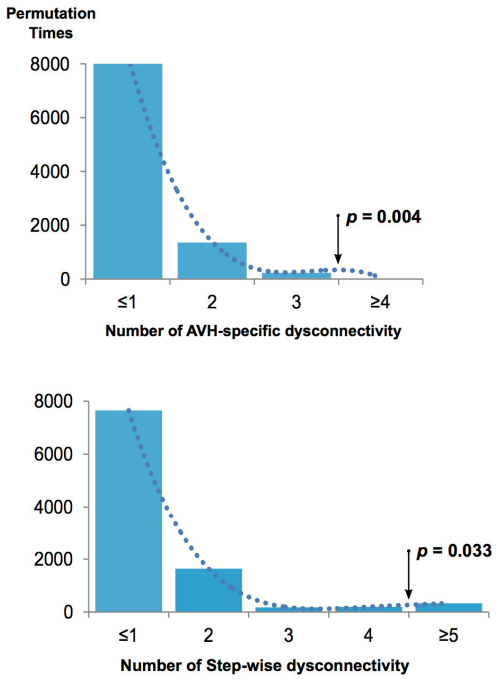
	CON	non-AVH	AVH	*p* value
AVH patients vs. Controls				
cdACC.R - INS.R	0.156	0.137	0.360	0.035
cdACC.R - STG.L	-0.104	-0.161	0.144	0.049
INS.R - STG.L	0.075	0.109	0.284	0.014
INS.R - IPL.L	0.210	0.244	0.449	0.024
cdACC.R - postC.R	0.106	0.282	0.392	0.006
cdACC.R - MTG.L	0.001	0.139	0.243	0.012
INS.R - STG.R	0.102	0.142	0.317	0.023
INS.R - IPL.R	0.190	0.219	0.399	0.035
pOper.L - postC.L	0.044	0.254	0.301	0.009
pOper.L - postC.R	0.047	0.167	0.255	0.021
postC.R - MTG.R	0.185	0.372	0.364	0.040
AVH patients vs. non-AVH patients				
cdACC.R - INS.R	0.156	0.137	0.360	0.020
cdACC.R - STG.L	-0.104	-0.161	0.144	0.014
INS.R - STG.L	0.075	0.109	0.284	0.043
INS.R - IPL.L	0.210	0.244	0.449	0.049
cdACC.R - STG.R	0.095	-0.001	0.335	0.010
cdACC.R - IPL.L	0.243	0.108	0.420	0.002
cdACC.L - IPL.R	0.301	0.179	0.402	0.013
cdACC.R - IPL.R	0.407	0.179	0.467	0.001
pTri.R – rsACC.R	0.063	0.168	-0.042	0.037

Abbreviations: L – left hemisphere; R – right hemisphere; STG - superior temporal gyrus; MTG - middle temporal gyrus; IPL - inferior parietal lobule; pTri – pars triangularis; pOper – pars opercularis; cdACC – caudal anterior cingulate cortex; rsACC – rostral anterior cingulate cortex; postC – postcentral gyrus; INS – insula

Supplementary Table 3 Jonckheere-Terpstra statistic on step-wise dysconnectivity*

	J-T statistic	*p* value
pOper.L – postC.L	3.762	0.0002
pOper.L – postC.R	3.460	0.0005
cdACC.R – postC.R	3.619	0.0003
INS.R – STG.L	3.190	0.0014
INS.R – IPL.L	3.159	0.0015

* Under the assumption of AVH > non-AVH > CON, $q < 0.05$, FDR correction for multiple comparison, corrected $p = 0.0016$



Supplementary Figure 1 Results of post-hoc analysis testing the specificity of selected ROIs. Here, 18 regions outside the predefined ROIs were randomly selected among all cortical areas. Functional connectivity among those random regions was tested for AVH-specific and step-wise effects as in the main analysis. For each iteration, the number of significant dysconnectivity was stored (x axis). This process was repeated 10,000 times to produce two probability distributions of both effects (y axis). Compared to the main results (four AVH-specific dysconnectivity and five step-wise effects), the probabilities of detecting equal or more AVH-specific or step-wise dysconnectivity in random regions were p = 0.004 and 0.033 respectively.

Distinct inter-hemispheric dysconnectivity in schizophrenia patients with and without auditory verbal hallucinations

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3

Abstract

Evidence from behavioral, electrophysiological and diffusion-weighted imaging studies suggest that schizophrenia patients have deficiencies in communication between the two hemispheres, and such abnormality may be related to the occurrence of auditory verbal hallucinations (AVH). To increase our understanding of aberrant inter-hemispheric communication in relation to AVH, we recruited 36 first-episode medication-naïve schizophrenia patients including one group of 18 patients with AVH symptoms, and another group of 18 patients without AVH symptoms, as well as 20 healthy controls. A T1-weighted magnetic resonance imaging (MRI) and resting-state functional MRI scans were acquired from all subjects to calculate homotopic inter-hemispheric connectivity (so called voxel-mirrored homotopic connectivity or VMHC). The VMHC measurement was compared across the three subject groups and entered into correlation analyses with patients' PANSS scores. We found that all patients showed reduced VMHC in the parahippocampal gyrus and higher VMHC in the striatum as compared to controls. Patients with AVH showed abnormal inter-hemispheric connectivity in areas belonging to the default mode network (DMN), inferior frontal gyrus and cerebellum, and patients without hallucinations exhibited VMHC alterations in the superior temporal gyrus and precentral gyrus. Moreover, patients' symptom scores correlated with inter-hemispheric connectivity in the anterior cingulate cortex, superior parietal lobule and precuneus gyrus. These results suggest that schizophrenia patients have inter-hemispheric connectivity abnormalities in multiple brain regions at the early stage of illness, and the localization of such abnormalities may be crucial to whether auditory verbal hallucinations develop.

Introduction

Schizophrenia is a debilitating neuropsychiatric disorder, which involves disrupted communication among brain areas (Friston and Frith, 1995; Stephan et al., 2009), including reduced inter-hemispheric connectivity (Guo et al., 2013; Lungu and Stip, 2012; Whitford et al., 2010). Homotopic inter-hemispheric connectivity is the strongest type of cortical-cortical functional interactions (Stark et al., 2008), which possibly reflects a strong tendency of functional coordination between the two hemispheres (Fox and Raichle, 2007). However, schizophrenia patients show reduced inter-hemispheric coherence as compared to healthy controls (Ribolsi et al., 2009). Behavioral and neurophysiological studies using lexical processing tasks showed that controls have advantage in processing words that presented in the bilateral visual fields than unilateral side, whereas patients failed to show such processing facilitation in recognizing bilaterally presented words, indicating a deficit in inter-hemispheric cooperation (Mohr et al., 2008, 2000). Diffusion tensor imaging (DTI) studies indicated that patients have aberrances in the corpus callosum (Lungu and Stip, 2012; Rotarska-Jagiela et al., 2008), which is the largest white matter fiber bundles in the brain and mediate information transfer mainly between the homotopic brain regions (Hofer and Frahm, 2006). Taken together, these studies suggest that schizophrenia involves abnormalities in the inter-hemispheric functional cooperation and its underlying structural substrate.

Abnormality of inter-hemispheric connectivity has been suggested to be particularly relevant to auditory verbal hallucinations (AVH) (Ke et al., 2010), which are experienced in 40% to 80% of patients diagnosed with schizophrenia (Aleman and Larøi, 2008). Theories of AVH suggest that the symptoms are associated with diminished lateralization in language related brain regions, which relies on proper communication between the two hemispheres (Gazzaniga, 2000; Hopkins and Rilling, 2000). For example, Bleich-Cohen et al. (2012) compared functional activation and connectivity in the inferior frontal gyrus (IFG) among patients with schizophrenia, patients with obsessive compulsive disorder and healthy controls. They found that decreased activation asymmetry and reduced inter-hemispheric connectivity in the inferior frontal gyrus only in schizophrenia patients, and that functional connectivity between the left and right inferior frontal gyrus correlated with the lateralization index of the inferior frontal gyrus. These results provide evidence that patients with AVH display diminished language related asymmetry, and that such abnormality may be related to aberrant inter-hemispheric connectivity.

To investigate intrinsic functional connectivity between the two hemispheres, we employed a newly developed measurement—voxel-mirrored homotopic connectivity (VMHC), defined as the functional connectivity between each voxel in one hemisphere and its mirrored counterpart in the other hemisphere. Using this index, Stark et al. (2008) revealed regional variations in inter-hemispheric connectivity, which is higher in the primary

sensorimotor regions than higher order brain areas. Zuo et al. (2010) revealed developmental trajectories of inter-hemispheric connectivity across the whole brain. In a range of neuropsychiatric disorders, including schizophrenia (Guo et al., 2014; Hoptman et al., 2012), autism (Anderson et al., 2011), major depressive disorder (Guo et al., 2013), mesial temporal lobe epilepsy (Xu et al., 2014), cocaine addiction (Kelly et al., 2011) and functional dyspepsia (Zhou et al., 2013), alterations in the VMHC index has been identified, suggesting that this measurement reflects inter-hemispheric connectivity changes with aging and diseases.

If AVH is related to language-related laterization abnormality, it is reasonable to speculate that inter-hemispheric connectivity may be different in schizophrenia patients suffering from AVH, as opposed to patients without AVH. In order to test this hypothesis, the current study compares inter-hemispheric functional connectivity among first-episode, medication-naïve schizophrenia patients with AVH, first-episode medication-naïve patients without AVH and healthy controls. The results will be informative in answering two questions: 1. Is there abnormal inter-hemispheric connectivity in early stage schizophrenia patients with no influence of anti-psychotics and prolonged illness duration? 2. If so, whether such alterations are presented in all patients, or specific to patients with AVH symptoms? Based on previous knowledge, we predict that patients with schizophrenia will exhibit aberrant inter-hemispheric connectivity. In addition, patients with and without AVH may demonstrate different connectivity deficits due to the involvement of inter-hemispheric connectivity in language related symptoms. This study will, for the first time, explore the origin of AVH in schizophrenia from the perspective of inter-hemispheric cooperation in resting-state fMRI.

Methods

Subjects

Thirty-six first-episode medication-naïve schizophrenia patients were recruited from the outpatient clinic at the Xijing Hospital, affiliated with the Fourth Military Medical University. Twenty healthy volunteers were recruited through advertisements. All participants were informed of the potential benefits and risks of this study and gave their written informed consent. The included participants were all right-handed native Chinese speakers, matched on age, gender and education level (Table 1). They were carefully examined to exclude the following conditions: a history of neurological disorder, severe medical disorders, substance abuse or dependence, prior electroconvulsive therapy or head injury resulting in loss of consciousness. This study was approved by the ethics committee of the Xijing Hospital, following the principles set forth by the Declaration of Helsinki.

Patients meeting diagnostic criteria for schizophrenia according to DSM-IV were assessed using the Positive and Negative Symptom Scale (PANSS Score ≥ 60) (Kay et al.,

1987). Two senior clinical psychiatrists performed the clinical-psychometric assessments with inter-rater reliability of higher than 90%. All patients were first-episode and antipsychotic-naïve at the time of scanning. The included patients were assigned to two groups according to the presence of AVH symptom. Those who reported AVH at least once a day for the past four weeks were assigned to AVH patient group. Patients who have never experienced AVH or have not experienced them within two years before recruitment were allocated to Non-AVH group. AVH patients were further evaluated using the Auditory Hallucination Rating Scale (AHRS) (Hoffman et al., 2003).

Table 1. Demographic and clinical characteristics of patients with AVH (*n* = 18), patients without AVH (*n* = 18), and healthy controls (*n* = 20)

Characteristics	AVH Patients	Non-AVH Patients	Controls
Age	22.56 ± 6.73	22.67 ± 3.80	23.43 ± 6.48
Sex (male/female)	10 / 8	9 / 9	11 / 9
Education (years)	12.44 ± 2.31	12.56 ± 2.19	13.43 ± 3.15
Duration of illness (months)	5.94 ± 5.93	12.44 ± 18.16	—
PANSS Total*	106.44 ± 13.55	88.06 ± 23.90	—
PANSS Positive*	31.11 ± 6.92	18.61 ± 8.68	—
PANSS Negative	25.78 ± 3.87	22.06 ± 10.43	—
PANSS General Psychopathology	49.56 ± 9.01	47.39 ± 9.84	—
AHRS Score	26.22 ± 8.10	—	—

Groups were matched for age, gender, and education

PANSS: Positive and Negative Syndrome Scale; AHRS: Auditory Hallucination Rating Scale

*Patients with AVH have significantly higher PANSS total and positive symptoms than patients without AVH.

Image acquisition

Magnetic resonance images were acquired on a 3.0-T Siemens Magnetom Trio Tim scanner. Participants were instructed to lie still in the scanner, keeping their eyes closed but do not falling asleep. They were judged to be awake at the start and conclusion of the scanning. Participants were scanned with a MRI-compatible head coil fitted with foam pads

and earplugs to minimize head motion and dampen scanner noise. Resting-state fMRI images and a high-resolution structural scan were acquired for each subject. The duration of the entire scanning process was about eight minutes.

Resting-state functional scans were acquired using a gradient-echo echo-planar imaging (EPI) sequence (TR = 2 s, TE = 30 ms, flip angle = 90°, FOV = 220 × 220 mm^2). Whole-brain volumes comprised 33 contiguous transverse slices with 4 mm thickness, 0.6 mm gap and 3.40 × 3.40 mm^2 in-plane resolution. For each participant, 240 whole-brain volumes were acquired, and the first ten volumes were discarded to allow for scanner equilibrium. T1-weighted structural images were obtained using a three-dimensional magnetization prepared rapid acquisition gradient echo (3D MPRAGE) sequence with the following parameters: TR = 2530 ms, TE = 3.5 ms, flip angle = 7°, FOV = 256 × 256 mm^2. The entire brain consisted of 192 slices, with 1 mm thickness, 0.5 mm gap.

Image Preprocessing

Data preprocessing was performed using the toolbox Data Processing Assistant for Resting-State fMRI (DPARSF) (Yan and Zang, 2010) based on MATLAB (Mathworks) platform. DPARSF provides an integrated pipeline for processing resting-state fMRI data. The preprocessing section starts with slice timing and then corrected for head motion. Data from two AVH patients were excluded from further analysis because of excessive head movement (translational > 2.0 mm and/or rotational > 2°). As recent findings suggest that "micro" head motions may also introduce group-related differences in resting-state fMRI metrics (Power et al., 2012; Satterthwaite et al., 2012; Van Dijk et al., 2012), group mean framewise displacement (FD) was compared among the remaining 54 participants (Jenkinson, 2002; Yan et al., 2013). The FD parameters did not differ significantly among the groups (F = 1.09, p = 0.35), and were further used as covariates in subsequent group comparisons. Several sources of spurious variance, including six rigid head motion parameters, signals averaged over the lateral ventricles and deep cerebral white matter, were removed using a linear regression model. After realignment and regression, functional images were normalized to their corresponding T1 image and resampled to 3 × 3 × 3 mm^3. The generated images were processed using spatial smoothing with a 4 mm full width at half maximum (FWHM) Gaussian kernel, linear detrend removal and temporally bandpass filtered (0.01 – 0.08 Hz).

Inter-hemispheric correlation

Coordination between the two hemispheres is an important feature of the brain functional architecture, which can be captured by resting-state connectivity between geometrically corresponding regions, i.e. the VMHC (Stark et al., 2008). This index has been

successfully used to quantify deficits in inter-hemispheric interaction in several brain disorders and in normal aging (Anderson et al., 2011; Hoptman et al., 2012; Kelly et al., 2011; Xu et al., 2014; Zuo et al., 2010). To account for geometric differences between hemispheres, the functional images were registered to a symmetric template through a three-step processing: First, the T1 images after segmentation (e.g., wco*.img or wco*.nii) were averaged across participants to generate a mean T1 image; then the mean T1 image is averaged with its flipped version to obtain a symmetrical T1 template; finally, individual T1 images were nonlinearly registered to the symmetrical T1 template and this transformation was applied to their functional data. The symmetrical functional images were used to calculate Pearson's correlation coefficients between all pair-wise voxels in the two hemispheres, resulting in a VMHC map for every participant. Correlation coefficients were converted to *z* scores (zVMHC) using Fisher *r*-to-*z* transformation for further statistical analyses.

Statistical analyses

Statistical analyses were performed using the REST toolbox on MATLAB platform (Song et al., 2011). We first performed the one-way ANOVA analysis comparing voxel-wise zVMHC across groups with head motion parameter as a covariate ($Z = 2.3$, cluster threshold at $p < 0.05$, GRF corrected), masked by the gray matter segmentation of the symmetric MNI template (thresholded at 40%) (Hoptman et al., 2012). Then, we averaged zVMHC values from a 5-mm sphere within each aberrant region identified from the one-way ANOVA analysis. The center of spheres was set at the peak coordinates of the clusters. Averaged zVMHC values of each sphere were compared across the three groups ($p < 0.05$, uncorrected).

Correlation analyses

As inter-hemispheric functional interaction has been suggested to be associated with symptom severity in schizophrenia patients (Bleich-Cohen et al., 2012), we performed correlation analyses between the PANSS total and subscale scores, and zVMHC values extracted from the 5-mm spheres of the aberrant regions in all patients. In patients with AVH, we further examined relationship between the ARHS score and zVMHC values in patients.

Results

Group difference in VMHC

Nine clusters showed significant differences in zVMHC values among the three groups (Figure 1). These regions included language processing brain areas: inferior frontal gyrus (peak coordinate: ±53, 18, 12), anterior cingulate cortex (peak coordinate: ±3, 54, 15),

posterior of superior temporal gyrus (peak coordinate: ±63, -42, 0), precentral gyrus (peak coordinate: ±42, -24, 63), precuneus cortex (peak coordinate: ±12, -60, 21), superior parietal lobule (peak coordinate: ±18, -69, 57), parahippocampal gyrus (peak coordinate: ±30, -27, -30); the striatum (peak coordinate: ±12, 15, 0) and anterior lobe of the cerebellum (peak coordinate: ±18, -30, -30).

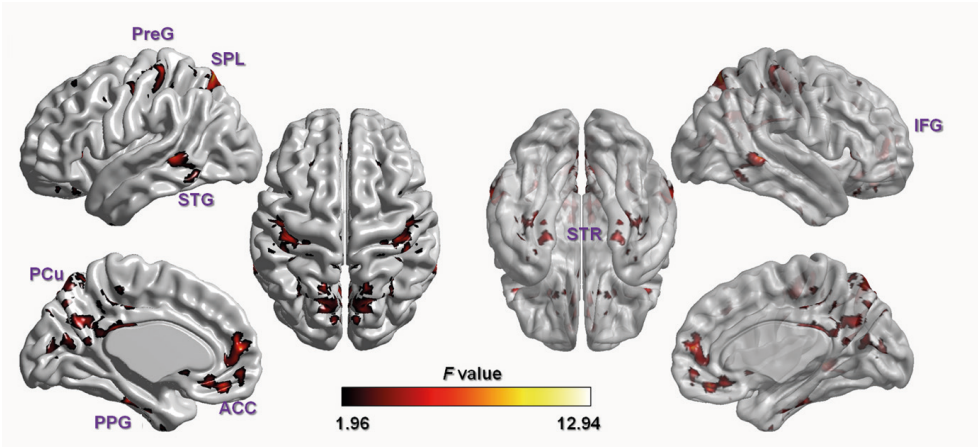

Figure 1 One-way ANOVA comparison on whole brain zVMHC map among the three groups of subjects ($Z = 2.3$, cluster threshold at $p < 0.05$, GRF corrected). Results were mapped on the ICBM152 brain template using BrainNet Viewer software (www.nitrc.org/projects/bnv/). The non-transparent left surfaces show sagittal views of the left hemisphere and an upper view of the brain. The right surface renderings show a bottom view of the brain and sagittal views of right hemisphere, with 60% opacity to show deeper structures. The cerebellum is not shown on this template.
Abbreviation: PreG: precentral gyrus; SPL: superior parietal lobule; STG: superior temporal gyrus; PCu: precuneus cortex; ACC: anterior cingulate cortex; PPG: parahippocampal gyrus; IFG: inferior frontal gyrus; STR: striatum.

According to the post hoc two-group comparisons, areas showing significant group differences from the one-way ANOVA analysis were classified into three categories: a). aberrances found in patients with AVH only, including inferior frontal gyrus, anterior cingulate cortex, precuneus cortex, superior parietal lobule and cerebellum; b). aberrances in patients without AVH only, including superior temporal gyrus and precentral gyrus; c). shared abnormalities by the two patient groups, comprising the parahippocampal gyrus and striatum (Figure 2, Table 2). Significant differences in the zVMHC measurement between AVH patients and Non-AVH patients were found in three regions: superior temporal gyrus,

anterior cingulate cortex and superior parietal lobule. Patients with AVH showed lower zVMHC values in these regions than patients without AVH, indicating that these abnormalities may relate to the different symptom profiles.

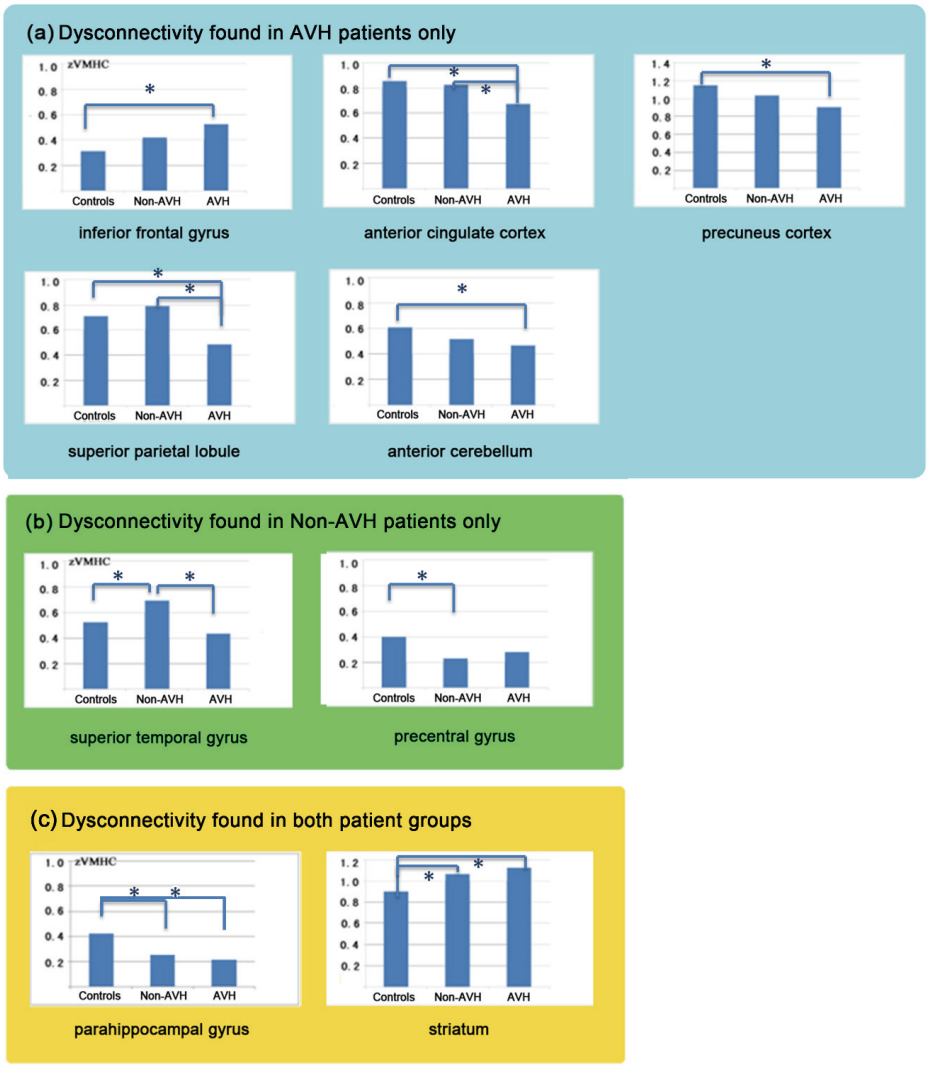

Figure 2 Inter-hemispheric connectivity differences between the two patient groups and healthy controls. Regional zVMHC values were extracted from a 5-mm sphere, with its center in the peak coordinates of the clusters identified from the one-way ANOVA analysis. (a) dysconnectivity only shown in AVH Patients (b) dysconnectivity only shown in Non-AVH Patients (c) common abnormalities in the two patient groups. Significant differences were marked with asterisks ($p < 0.05$, uncorrected).

Table 2 Post-hoc two-group comparisons of regions identified from the ANOVA analysis

Brain areas	zVMHC values of ROI			*F*	Post hoc *p* values		
	Con	Non-AVH	AVH		Con vs. AVH	Con vs. Non-AVH	AVH vs. Non-AVH
Dysconnectivity found in AVH patients only							
inferior frontal gyrus	0.31	0.42	0.52	4.03	**0.02***	0.44	0.53
anterior cingulate cortex	0.86	0.83	0.67	6.24	**0.004***	1.00	**0.03***
precuneus cortex	1.15	1.04	0.90	6.37	**0.002***	0.31	0.18
superior parietal lobule	0.71	0.79	0.49	9.51	**0.007***	0.74	**0.000***
anterior cerebellum	0.61	0.52	0.45	3.22	**0.05***	0.40	0.96
Dysconnectivity found in Non-AVH patients only							
superior temporal gyrus	0.54	0.75	0.46	7.97	0.98	**0.01***	**0.001***
precentral gyrus	0.40	0.23	0.28	5.03	0.12	**0.01***	1.00
Dysconnectivity found in both patient groups							
parahippocampal gyrus	0.42	0.25	0.21	5.62	**0.01***	**0.04***	1.00
striatum	0.90	1.06	1.12	6.33	**0.004***	**0.04***	1.00

*Significant differences in post hoc tests are marked in bold type with an asterisk, $p < 0.05$. AVH: patients with auditory verbal hallucinations, Non-AVH: patients without AVH, Con: control subjects

Correlation analysis

Correlations with PANSS scores in all patients – Significant negative correlations were found between anterior cingulate cortex zVMHC values and PANSS positive, negative, and total scores (Figure 3a, 3b, 3c). The superior parietal lobule zVMHC showed a significant relationship with PANSS positive score (Figure 3d), and the zVMHC value in the precuneus cortex negatively correlated with PANSS general psychopathology score (Figure 3e).

Correlations with AHRS scores in AVH patients – No significant correlations with AHRS scores were found in the AVH patients. Table S1 describes the associations between

zVHMC values of each aberrant region detected in the one-way ANOVA, and AHRS scores. No significant correlation was identified between inter-hemispheric connectivity and AHRS scores.

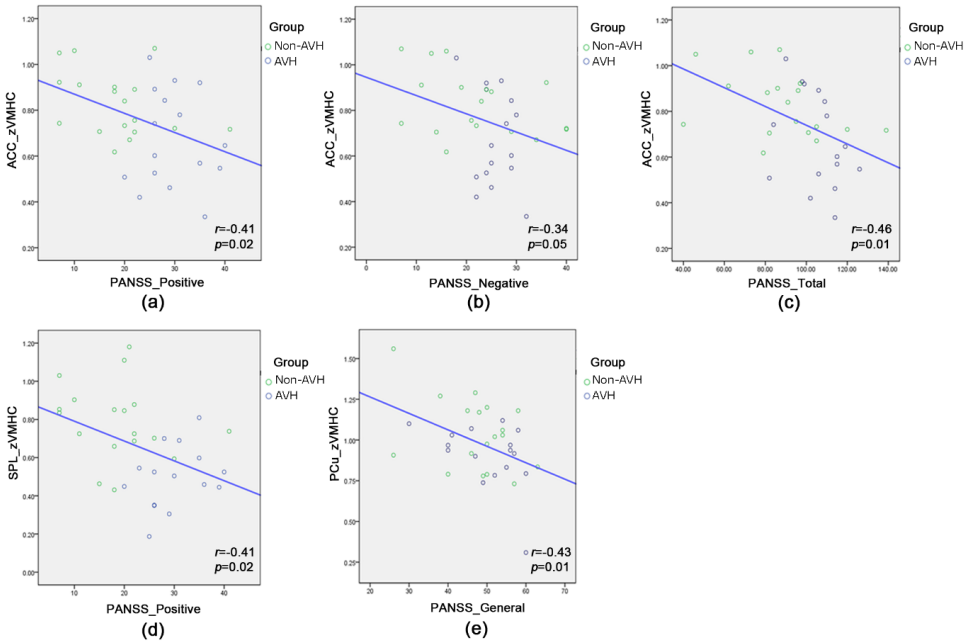

Figure 3 Correlation analyses between PANSS scores and zVMHC values in patients. (a-c) the anterior cingulate cortex zVMHC correlates with PANSS positive, negative and total scores (d) superior parietal lobule zVMHC associates with PANSS positive symptoms (e) precuneus cortex zVMHC correlates with PANSS general psychopathology.

Discussion

The current study examined whole brain inter-hemispheric connectivity in first-episode, medication-naïve schizophrenia patients with AVH symptoms, patients without AVH symptoms and healthy controls. Our results show that patients with and without AVH are characterized by distinct inter-hemispheric connectivity abnormalities, with shared connectivity alterations in the parahippocampal gyrus and striatum. In addition, inter-hemispheric connectivity in the anterior cingulate cortex, superior parietal lobule and precuneus cortex are found to correlate with patients' PANSS scores. Our findings are in line with behavioral, electrophysiological and task-based fMRI studies showing abnormal functional cooperation between the two hemispheres in schizophrenia patients (Mohr et al.,

2008, 2000), and extends these findings by showing distinct inter-hemispheric connectivity abnormalities in patients with AVH as compared to those without AVH symptoms.

Patients with AVH symptoms exhibited widespread inter-hemispheric connectivity changes, including a higher level of connectivity between the bilateral inferior frontal gyrus as compared to controls, with Non-AVH patients showing intermediate values. The left inferior frontal gyrus (Broca's area) is responsible for speech production, and schizophrenia patients have been suggested to show reduced lateralization in the inferior frontal gyrus (Bleich-Cohen et al., 2009). Importantly, a previous study found an correlation between task-induced activation asymmetry and inter-hemispheric connectivity in the inferior frontal gyrus (Bleich-Cohen et al., 2012), suggesting that disease-related asymmetry loss may associate with connectivity alterations in schizophrenia. Previous research has also demonstrated the involvement of the inferior frontal gyrus in emergence of hallucinations (Hoffman et al., 2007; Kompus et al., 2011; Steinmann et al., 2014). Hoffman et al., (2011) observed greater activation in the left inferior frontal gyrus occurred before onset of hallucinations in schizophrenia patients, and a stronger functional coupling between the left inferior frontal gyrus and right temporal regions in hallucination patients as compared to patients without hallucinations. Raij et al., (2009) reported an elevated activation in the inferior frontal gyrus accompanying with AVH occurrence. In the current study, we didn't find a significant relationship between patients' symptom scores and zVMHC value in the inferior frontal gyrus. A possible explanation is that psychotic symptoms may be more closely related to connectivity between the inferior frontal gyrus and other brain regions rather than inter-hemispheric connectivity.

Patients with AVH symptoms demonstrated abnormal inter-hemispheric connectivity in the anterior cingulate cortex, precuneus cortex and superior parietal lobule, which are considered as parts of the default mode network (DMN). The DMN comprises a set of brain regions that maintain a high level of neural activity during rest, and deactivated during goal-oriented performance (Fransson, 2006; Greicius et al., 2003; Masawaki et al., 1988). The DMN has been associated with internal thoughts (Andrews-Hanna, 2012; Christoff et al., 2009; Gerlach et al., 2011), and self-referential processing (D'Argembeau et al., 2005; Raichle and Snyder, 2007). According to previous work, disruptions of DMN activity may lead to positive symptoms of schizophrenia (Whitfield-Gabrieli et al., 2009), and especially AVH symptoms (Allen et al., 2012; Jones, 2010). Northoff et al., (2011) postulated a "resting state hypothesis", suggesting that AVH symptoms may result from abnormal connectivity between the auditory cortex and regions comprising the DMN. The involvement of DMN abnormalities in schizophrenia symptomology is supported by our results showing associations between inter-hemispheric connectivity of DMN regions and PANSS total and

subscale scores. In addition, we suggested that inter-hemispheric connectivity of DMN regions are more severely affected in AVH patients than Non-AVH patients.

The cerebellum has traditionally been regarded as a brain structure that is exclusively involved in body balance and muscular coordination, but there is increasing recognition for its putative role in cognitive and affective functions and pathological involvement in schizophrenia (Collin et al., 2011; Strick et al., 2009). Two previous studies reported decreased cerebellar VMHC in schizophrenia patients (Guo et al., 2014; Hoptman et al., 2012). In the current study, we found a lower inter-hemispheric connectivity in the cerebellum in AVH patients and a trend-level lower VMHC in Non-AVH patients, suggesting that lower inter-hemispheric connectivity in the cerebellum may be a general deficiency in patients, but most severely changed in patients with AVH symptoms.

In the current study, we found that Non-AVH patients showed a higher zVMHC value in the superior temporal gyrus as compared to AVH patients and controls, whereas a previous study reported lower VMHC values in a number of regions including the superior temporal gyrus (Guo et al., 2014). Using the same measurement, Hoptman et al., (2012) did not find connectivity alterations in the superior temporal gyrus in schizophrenia patients. A possible explanation for the inconsistence between studies is that inter-hemispheric connectivity alteration in the superior temporal gyrus may relate to symptom profiles of recruited subjects and thus differ between studies.

The two patient groups consistently showed significant connectivity alterations in the striatum and parahippocampal gyrus, suggesting that these abnormalities may participate in the general pathological changes of schizophrenia. The striatum is a crucial component of dopaminergic pathway, and malfunction of this area has been related to emergence of positive symptoms in schizophrenia (Kapur et al., 2005). The parahippocampal gyrus and nearby brain regions are known for their functional roles in episodic memory processing (van Strien et al., 2009). Therefore, connectivity disruptions in the parahippocampal gyrus may result in cognitive impairments in schizophrenia (Achim and Lepage, 2005).

There are several limitations to this study that warrant further consideration in future research. First, as a newly developed measurement, the physiological implication of VMHC index has not been clearly clarified. Unlike structural connectivity that is generally decreased in patients, functional connectivity has been found to exhibit bi-directional changes, making it difficult to distinguish between primary decrease and secondary compensatory mechanisms. Previous studies have shown regional variation of VMHC in different brain regions (Stark et al., 2008) and throughout the lifespan of healthy subjects (Zuo et al., 2010). Yet biological origin of VMHC and its relationship with structural connectivity remains to be explored. Furthermore, to calculate VMHC, subjects' functional images were registered to a group averaged symmetric structural template, to deal with the morphologic asymmetry of the

brain. This could be improved by developing more advanced algorithms to define functional homotopy (for example, clustering inter-hemispheric voxels based on their resting-state connectivity) in future studies (Kelly et al., 2011). Second, the strict criterion to recruit medication-naïve, first-episode patients limited the sample size of this study. On the other hand, this inclusion criterion has presumably minimized the influences of medication, cohort effects and illness-related environmental factors. Future studies with a larger sample size will be informative in detecting more subtle changes.

The present study indicates shared abnormalities of inter-hemispheric connectivity in the striatum and parahippocampal gyrus in all patients with schizophrenia. The AVH patients exhibited significant alterations in the inferior frontal gyrus, cerebellum and several DMN regions, whereas Non-AVH patients showed different abnormalities in the superior temporal gyrus and precentral gyrus. Moreover, inter-hemispheric functional connectivity disturbances of DMN regions negatively correlated with PANSS scores in all patients. The current study corroborates prior findings on abnormal inter-hemispheric connectivity in schizophrenia patients, and further extends previous findings by showing distinct connectivity patterns in patients with and without AVH, indicating dissociable neural mechanisms may exist in patients with different behavioral symptoms.

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Author contributions

HY, DMM, YBX, and HNW designed the study. QRT, HNW, and MX enrolled the patients. HY, YBX, LBC, KL, and SQ collected the MRI data. JBS, YQZ, XC, and YBX analyzed the data. XC, HNW, YBX, and LBC involved in manuscript drafting. PH, GC, XC, LBC, and YBX revised the manuscript. All authors reviewed the manuscript.

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Supplementary material of

Distinct inter-hemispheric dysconnectivity in schizophrenia patients with and without auditory verbal hallucinations

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Table S1 Correlation between zVMHC values and ARHS scores
(Signals were extracted from a 5-mm sphere of the aberrant regions on zVMHC map)

		IFG	ACC	PCU	SPL	CEL LUM	STG	PreG	PPG	STR
Hoffman Score	***r***	-0.36	-0.35	-0.19	0.35	-0.03	0.01	-0.48	0.36	-0.49
	p	0.18	0.19	0.48	0.18	0.91	0.97	0.06	0.18	0.06

Abbreviation: IFG: inferior frontal gyrus; ACC: anterior cingulate cortex; PCu: precuneus cortex; SPL: superior parietal lobule; CELLUM: cerebellum; STG: superior temporal gyrus; PreG: precentral gyrus; PPG: parahippocampal gyrus; STR: striatum.

Interhemispheric connectivity and hemispheric specialization in schizophrenia patients and their unaffected siblings

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Abstract

Hemispheric integration and specialization are two prominent organizational principles for macroscopic brain function. Impairments of interhemispheric cooperation have been reported in schizophrenia patients, but whether such abnormalities should be attributed to effects of illness or familial risk remains inconclusive. Moreover, it is unclear how abnormalities in interhemispheric connectivity impact hemispheric specialization. To address these questions, we performed magnetic resonance imaging (MRI) in a large cohort of 253 participants, including 84 schizophrenia patients, 106 of their unaffected siblings and 63 healthy controls. Interhemispheric connectivity and hemispheric specialization were calculated from resting-state functional connectivity, and compared across groups. Results showed that schizophrenia patients exhibit decreased interhemispheric connectivity as compared to controls (p = 0.04) and siblings (p = 0.004). In addition, patients showed (marginally) higher levels of hemispheric specialization as compared to siblings (p = 0.01) and controls (p = 0.10). Level of interhemispheric connectivity and hemispheric specialization correlated with duration of illness in patients (p = 0.03, 0.049 respectively). No significant alterations were identified in siblings relative to controls on both measurements. Furthermore, alterations in interhemispheric connectivity correlated with changes in hemispheric specialization in patients relative to controls (p < 0.001) and siblings (p < 0.001). Taken together, these results suggest that decreased interhemispheric connectivity and associated abnormalities in hemispheric specialization are features of established illness, rather than an expression of preexistent familial risk for schizophrenia.

Keywords: schizophrenia; unaffected relative; interhemispheric connectivity; hemispheric specialization; resting-state fMRI.

Introduction

Two prominent features of macroscopic brain organization are hemispheric integration, characterized as a high level of collaboration between bilateral brain regions, and hemispheric specialization, referring to the association of behavioral traits with a particular side of brain (Hervé et al., 2013; Serrien et al., 2006). Proper integration and segregation of the two cerebral hemispheres are crucial for a broad repertoire of cognitive functions including language, visuospatial attention, and manual preference (Cai et al., 2013; Gotts et al., 2013; Liu et al., 2009). Conversely, disturbances of hemispheric coordination may impact cognitive and behavioral functioning (Gazzaniga, 1995; Toga and Thompson, 2003) and contribute to neuropsychiatric disorders including schizophrenia (Bleich-Cohen et al., 2012; Crow, 1998; Ribolsi et al., 2009; Sommer et al., 2001).

Multiple lines of evidence suggest that schizophrenia may involve abnormalities in interhemispheric connectivity and cooperation, which may contribute to central features of the illness such as auditory verbal hallucinations (Chang et al., 2015) and cognitive deficits (Liu et al., 2018). Behavioral studies indicate that a bilateral processing advantage that is normally present during language tasks is compromised in schizophrenia patients (Lohr et al., 2006; Mohr et al., 2000) and studies using event-related potentials (ERP) show evidence of impaired information transfer between the hemispheres during cognitive tasks in schizophrenia (Barnett and Kirk, 2005; Endrass et al., 2002; Mohr et al., 2008). Moreover, structural neuroimaging studies report that schizophrenia patients have decreased volume and fiber integrity of the corpus callosum, the major fiber bundle connecting the two hemispheres (Hulshoff Pol et al., 2004; Knöchel et al., 2012; Kubicki et al., 2008; Patel et al., 2011), and fMRI studies show impaired levels of functional synchronization between homotopic brain regions (i.e., corresponding areas in the left and right hemisphere) at rest (Hoptman et al., 2012; Li et al., 2015).

However, important open questions regarding interhemispheric connectivity in schizophrenia remain. First, it is unclear whether interhemispheric connectivity deficits are primarily related to the effects of the illness or a reflection of (genetic) risk for the disorder (Boos et al., 2012; MacDonald et al., 2009). Unaffected first-degree relatives of schizophrenia patients are a valuable population to study this question as they share the genetic predisposition and environmental factors for the illness, but are not clinically affected. Previous studies examining interhemispheric connectivity suggest that unaffected relatives exhibit subtle structural (Knöchel et al., 2012) and functional (Guo et al., 2014) connectivity impairments relative to healthy controls. However, no previous study has compared functional interhemispheric connectivity across unaffected relatives, their affected probands and healthy controls. In the current study, we examine interhemispheric functional

connectivity in a large cohort of schizophrenia patients and their nonpsychotic siblings to assess how interhemispheric connectivity alterations relate to risk for schizophrenia.

Another open question is what the consequence of reduced interhemispheric connectivity may be for overall brain function. We hypothesize that impaired interhemispheric connectivity may cause the two hemispheres to function more autonomously, favoring within over between-hemispheric interregional connectivity. To test this hypothesis, we examine how interhemispheric connectivity deficits in schizophrenia patients and their unaffected siblings relate to hemispheric specialization as quantified by the hemispheric autonomy index (Gotts et al., 2013; Mueller et al., 2015; Wang et al., 2014).

Material and methods

Participants

A total of 253 participants, including 84 schizophrenia patients, 106 of their unaffected siblings and 63 healthy controls, were recruited at the University Medical Center Utrecht between September 2004 and April 2008, as part of the Genetic Risk and Outcome of Psychosis (GROUP) study (Korver et al., 2012). The GROUP study was conducted by four university psychiatric centers and their affiliated mental health care institutions in the Netherlands. The medical ethics committee of the University Medical Center Utrecht approved the current study. All subjects provided written informed consent prior to participation.

For all participants, presence or absence of current and lifetime psychopathology was established using the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992). This semi-structured interview is designed to obtain comprehensive information on current and past signs and symptoms of major psychiatric disorders, premorbid functioning, sociodemographic status, treatment, and course of illness. Patients were included if they met Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) criteria for schizophrenia or schizoaffective disorder. Symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). The dosage of antipsychotic medication patients were taking at the time of scanning were converted to a chlorpromazine equivalent dosage (Kroken et al., 2009).

Patients and siblings originated from 120 unique families and included 48 patient-sibling pairs or triplets. The control group comprised 63 subjects from 57 families. A family identification code (family ID) was assigned to every subject to keep track of their pedigrees. Siblings and healthy controls were excluded for any current or previous psychotic disorder. In addition, controls were examined with the Family Interview for Genetic Studies (Maxwell, 1992), to exclude a family history of psychotic disorders (in first- or second-degree relatives). Exclusion criteria for all participants included a history of head trauma or major medical or

neurological illness. All participants were between 18 and 60 years of age at the time of inclusion. Demographics and clinical information are described in Table 1.

Table 1 Demographic and clinical information of the three groups of participants (controls, siblings, schizophrenia N = 63, 106, 84)

	Controls	**Siblings**	**Patients**	***p* value** [a]
Age	29.16 ± 7.59	29.50 ± 7.65	29.86 ± 5.27	0.83
Sex (M / F)	29 / 34	49 / 57	70 / 14	<0.001
IQ	113.76 ± 15.66	105.96 ± 17.10	95.99 ± 15.21	<0.001
Handedness (right / left / mix)	44 / 8 / 1	94 / 6 / 6	70 / 5 / 4	0.23
Illness duration (years) [b]	--	--	5.91 (4.12)	--
Chlorpromazine dosage (mg/day)	--	--	300 (200, 600)	--
PANSS total	--	--	48.38 ± 14.89	--
PANSS positive	--	--	11.58 ± 5.42	--
PANSS negative	--	--	12.23 ± 4.35	--
PANSS general psychopathology	--	--	24.57 ± 7.05	--

[a] Group comparison of continuous variables (Age, IQ) are tested with one-way analysis of variance (ANOVA). Nominal variables (sex, handedness) were compared using chi-square statistics.

[b] Distribution of illness duration and medication is skewed, therefore median and inter-quartile range was reported.

Data acquisition

MRI scans were acquired on two 1.5 T Philips Achieva scanners at the University Medical Center Utrecht, with the same acquisition protocol. The scanner information was included as a covariate in further group-comparisons. For each subject, resting-state functional magnetic resonance imaging (fMRI) time series were acquired with a three dimensional (3D) PRESTO acquisition scheme. The acquisition parameters were: TR / TE = 21.1 / 31.1 ms; flip angle =

90°; field of view = 256 × 256 mm^2, voxel size = 4 × 4 × 4 mm^3, 900 time frames, consisting of 32 slices covering whole brain. In addition, a T1-weighted scan was collected as anatomical reference with the following scanning parameters: TR/TE = 30 / 4.6 ms; flip angle = 30°; field of view = 256 × 256 mm^2, voxel size = 1 × 1 × 1.2 mm^3.

Data preprocessing

Resting-state fMRI data were preprocessed using the Data Processing and Analysis for Brain Imaging (DPABI) toolbox (Yan et al., 2016) on MATLAB v. R2016a (Mathworks). For every subject, the first 10 functional volumes were removed to allow for signal stabilization. The rest of the functional images were realigned, co-registered with their individual anatomic image, and corrected for nuisance variables (six rigid realignment parameters, signal drift, averaged signal from white matter and cerebrospinal fluid) using a linear regression model. Band-pass filtering (0.01 - 0.1 Hz) was applied to fMRI time-series to reduce effects of low-frequency drifts and high-frequency noise. Subsequently, the functional scans were spatially normalized to MNI space using a symmetric group-specific T1 template. Spatial smoothing was applied to normalized images with a 4 mm full-width half-maximum (FWHM) Gaussian kernel. In view of recent findings on the influence of in-scanner head motion on fMRI signals (Power et al., 2012; Satterthwaite et al., 2013; Yan et al., 2013), images with excessive movement (frame-wise displacement > 0.5 mm) and 1 backward and 2 forward scans relative to the marked frame, were scrubbed (Power et al., 2012). The scrubbing procedure negated group-differences in head motion (before and after scrubbing p = 0.04, 0.13, Supplementary Table 1).

The Harvard-Oxford atlas (Goldstein et al., 2007; Makris et al., 2006) was applied to whole-brain functional data in order to parcellate the brain into 112 bilateral regions (48 cortical and 8 subcortical regions per hemisphere). Region names, abbreviations, anatomic classification (frontal, temporal, parietal, occipital lobe, cingulate cortex and subcortical regions) and functional hierarchy (primary sensorimotor regions, unimodal, heteromodal association areas, (para)limbic and subcortical regions) are referenced in Supplementary Table 2. The functional hierarchy subdivision scheme is based on previous studies (Bassett et al., 2008; Mesulam, 1998; Stark et al., 2008). Regional time-series were averaged across voxels, and cross-correlated for each pair of brain regions using Pearson's correlation. Correlation coefficients were converted to z scores using Fisher's z transformation, serving as a measure of functional connectivity strength between two regions.

Interhemispheric connectivity

The level of functional connectivity between 56 regions in one hemisphere and their corresponding areas in the other hemisphere is used here as the measure of

interhemispheric connectivity. Interhemispheric connections between homotopic regions are among the strongest functional associations in cortical-cortical interactions (Stark et al., 2008) and have reliably been identified in both healthy and clinical populations (Anderson et al., 2011; Guo et al., 2013; Wei et al., 2014; Zhou et al., 2013). As a supplementary analysis, group-differences in heterotopic interhemispheric connectivity and left and right intrahemispheric connectivity were examined in the same manner as homotopic interhemispheric connectivity. Moreover, to assess the consistency of interhemispheric connectivity measurements between studies, we performed an additional analysis examining the correlation in regional interhemispheric connectivity in controls between this study and the study of Stark et al., (2008).

Hemispheric specialization

Hemispheric specialization is calculated here as the difference in connectivity strength between intrahemispheric and heterotopic interhemispheric connections of each brain region. Homotopic interhemispheric connections were not used in the calculation of hemispheric specialization so that we could examine relationship between hemispheric specialization and interhemispheric connectivity in the following analysis. A positive value of hemispheric specialization indicates that a region or hemisphere is preferentially connected to other regions within its own hemisphere, whereas a negative value suggests that a region or hemisphere is more strongly connected to regions in the contralateral hemisphere. The method used to compute hemispheric specialization using intrinsic functional connectivity has been described in previous studies using a voxel-based analysis (Mueller et al., 2015; Wang et al., 2014).

Statistical analysis

Effects of group and covariates on interhemispheric connectivity and hemispheric specialization were assessed using linear-mixed effect models. For interhemispheric connectivity, fixed-effects include: group status, age, sex, handedness, scanner and head motion. To control for potential group-differences in global connectivity, mean connectivity strength of all non-homotopic connections (i.e., all intrahemispheric and heterotopic interhemispheric connections) was included as a fixed-effect term (global FC). Family ID, encoding the subjects' pedigree information, was included as random-effect term to control for family ties between subjects (Equation 1).

Equation 1:

$$Interhemispheric\ connectivity_i \sim 1 + \beta_1 \times Group_i + \beta_2 \times Age_i + \beta_3 \times Sex_i + \beta_4 \times Handedness_i + \beta_5 \times Scanner_i + \beta_6 \times Motion_i + \beta_7 \times GlobalFC_i + (1|FamilyID_i) + \varepsilon_i$$

For hemispheric specialization, group status, age, sex, handedness, scanner and head motion were again included as fixed-effect terms. Additionally, we included hemisphere as a fixed factor to examine potential differences in connectivity patterns between the two hemispheres. Random effect terms include family ID and subject ID nested within family ID. Equation 2:

$$Hemispheric\ specialization_i \sim 1 + \beta_1 \times Group_i + \beta_2 \times Age_i + \beta_3 \times Sex_i + \beta_4 \times Handedness_i + \beta_5 \times Scanner_i + \beta_6 \times Motion_i + \beta_7 \times Hemisphere_i + (1|FamilyID_i) + (1|SubjectID_i{:}\ FamilyID_i) + \varepsilon_i$$

Linear-mixed effects analysis was first applied to overall interhemispheric connectivity and hemispheric specialization ($p < 0.05$), and then to each of 56 regional measurements, controlling for multiple comparisons (Bonferroni correction, $p < 0.05/56$). Overall and regional measurements with significant group effects were subjected to post-doc bivariate comparisons between each pair of subject groups ($p < 0.05$).

Relationship between interhemispheric connectivity and hemispheric specialization

To test whether schizophrenia-related changes in interhemispheric connectivity co-vary with alterations in hemispheric specialization, we calculated the mean difference between controls and patients on both measurements for each brain region. Pearson's correlation analysis was performed on group-differences of interhemispheric connectivity and hemispheric specialization across regions ($p < 0.05$). The same analysis was performed for siblings as compared to patients, and for controls compared to siblings.

Correlation between connectivity alteration and clinical factors

To examine the potential clinical relevance of connectivity changes in patients, the level of interhemispheric connectivity and hemispheric specialization were correlated with illness duration and PANSS total scores in patients ($p < 0.05$). Moreover, antipsychotic medication was examined as a potential confounder of functional connectivity measurements by correlating daily medication in chlorpromazine equivalent dose with interhemispheric connectivity and hemispheric specialization.

Results

Interhemispheric connectivity

Group mean homotopic interhemispheric connectivity strength was plotted on a brain surface for each subject group (Figure 1). Regional variation in interhemispheric connectivity indicated higher bilateral synchrony in primary sensorimotor, auditory cortex and midline regions as compared to heteromodal association cortex. Regional interhemispheric

connectivity in healthy controls correlated with values reported in a previous study (Stark et al., 2008) in healthy subjects (r = 0.41, p = 0.002, Supplementary Figure 1).

Linear mixed-effects analysis showed a significant main effect of group on homotopic interhemispheric connectivity (F = 4.31, p = 0.01, Figure 1), such that interhemispheric connectivity was lower in patients as compared to both controls (p = 0.04) and siblings (p = 0.004). Group-differences for heterotopic interhemispheric connectivity or left and right intra-hemispheric functional connectivity was not significant after controlling for global connectivity differences (F = 0.61, 0.11, 0.14, p = 0.54, 0.89, 0.87 respectively, Supplementary Figure 2).

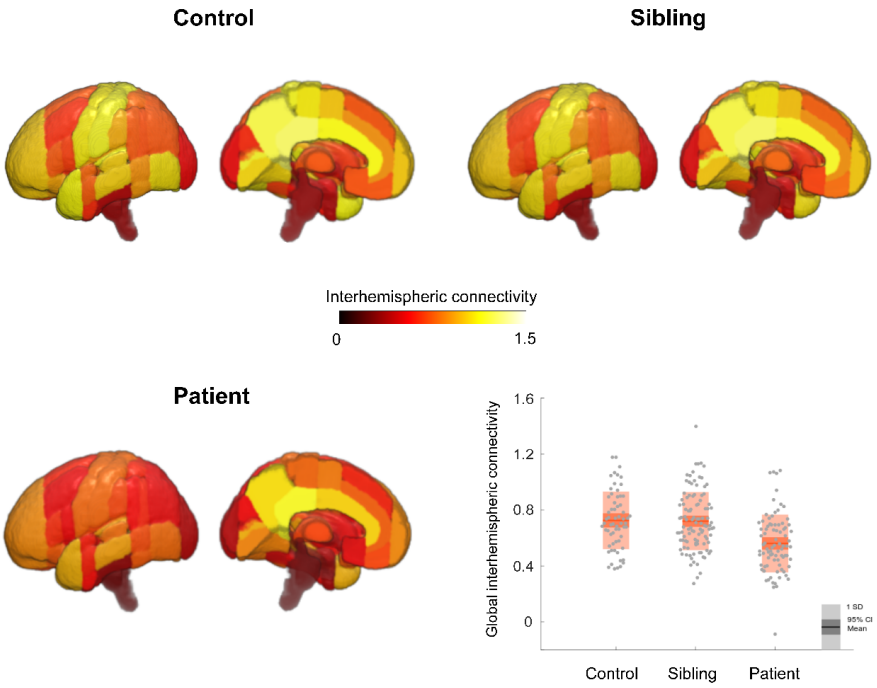

Figure 1 Regional homotopic interhemispheric connectivity strength plotted for three subject groups. The bar plot on the lower right part of the figure depicts global mean interhemispheric connectivity for every subject. Patients had lower interhemispheric connectivity strength than controls (p = 0.04) and siblings (p = 0.004).

Regional homotopic interhemispheric connectivity showed a significant group-effect in pre- and postcentral gyrus, subcallosal cortex and anterior supramarginal gyrus (all p < 0.05/56, Figure 2). Post-hoc analyses revealed significant reductions in interhemispheric

connectivity in patients relative to both controls and siblings for all these regions ($p < 0.05$). There were no significant group-differences in regional interhemispheric connectivity between controls and siblings.

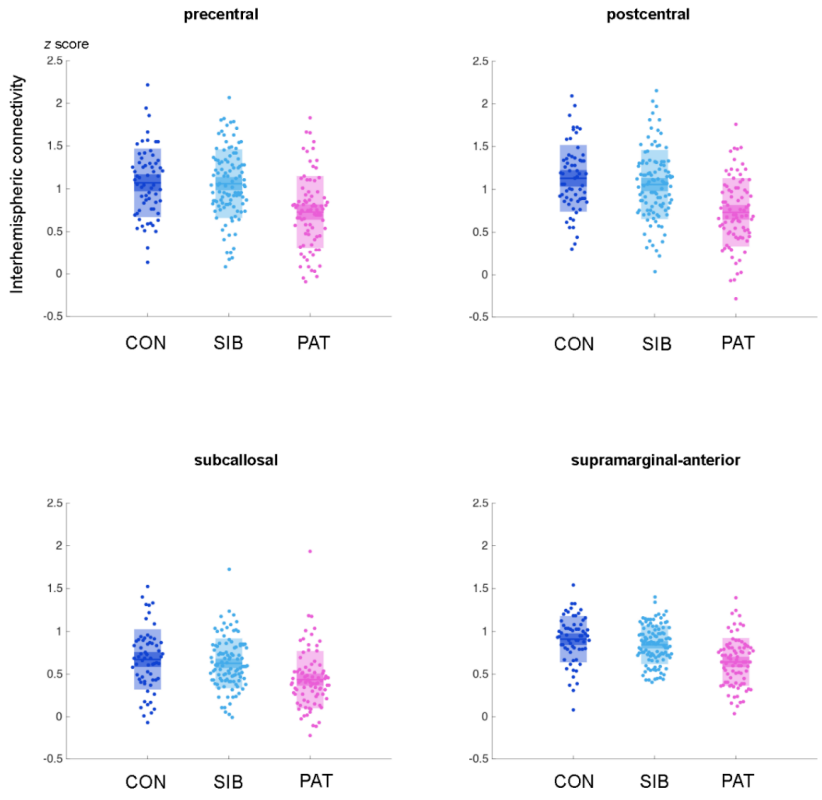

Figure 2 Regions showing a significant group effect on interhemispheric connectivity (Bonferroni corrected, $p < 0.05/56$). Post-hoc analyses revealed significant reductions in interhemispheric connectivity in patients relative to both controls and siblings for all these regions ($p < 0.05$). Abbreviation: CON, healthy controls; SIB, unaffected siblings; PAT, schizophrenia patients.

Hemispheric specialization: group effects

Regional variation of hemispheric specialization showed a distinct pattern from interhemispheric connectivity (Figure 3). Heteromodal regions showed higher levels of hemispheric specialization than primary sensorimotor areas, indicating a tendency of heteromodal regions to interact preferentially with ipsilateral brain areas.

There was a significant main effect of group on global hemispheric specialization (F = 3.73, p = 0.02). Post-hoc comparisons showed that hemispheric specialization is higher in patients as compared to siblings (p = 0.01), and marginally higher relative to controls (p = 0.10), indicating that functional connectivity in schizophrenia patients is more confined within their own hemispheres.

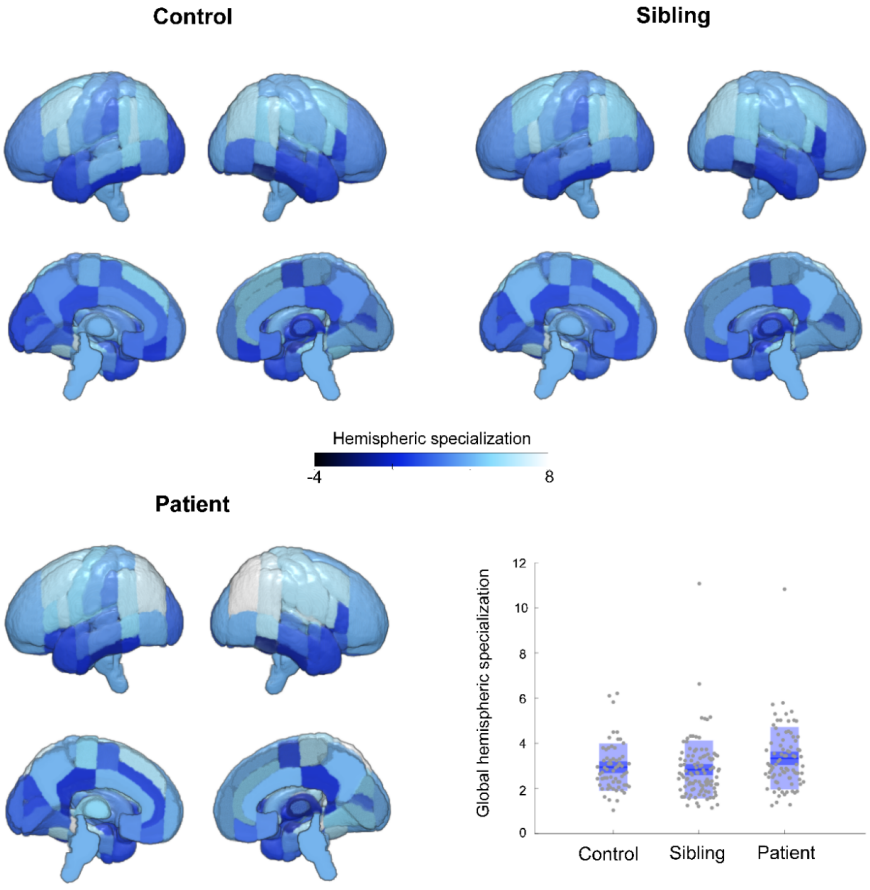

Figure 3 Regional hemispheric specialization in three subject groups. Patients with schizophrenia had higher global hemispheric specialization than siblings (p = 0.01), and marginally higher hemispheric specialization than controls (p = 0.10).

Ten out of 56 whole-brain areas showed significant group effects of regional hemispheric specialization (p < 0.05/56), including parietal, temporal and occipital association cortices, sensorimotor regions and limbic areas, mainly belonging to unimodal and heteromodal association cortices (Figure 4A). In these regions, hemispheric

specialization was increased in patients as compared to both controls and siblings ($p < 0.05$). There were no significant group-differences between siblings and controls in hemispheric specialization.

Hemispheric specialization: hemispheric effects

There were no significant hemispheric effects of global hemispheric specialization ($p = 0.89$). On the regional level, significant main effects of hemisphere were observed for a range of cortical and subcortical brain regions ($p < 0.05/56$) (Figure 4B). Higher left-hemispheric specialization was found predominantly in superior/inferior frontal and inferior/middle temporal gyrus, as well as subcortical regions and posterior cingulate cortex. Regions with higher right-hemispheric specialization included parietal and occipital areas, central opercular cortex, heschls gyrus, planum temporale, caudate nucleus and accumbens, anterior and paracingulate gyrus.

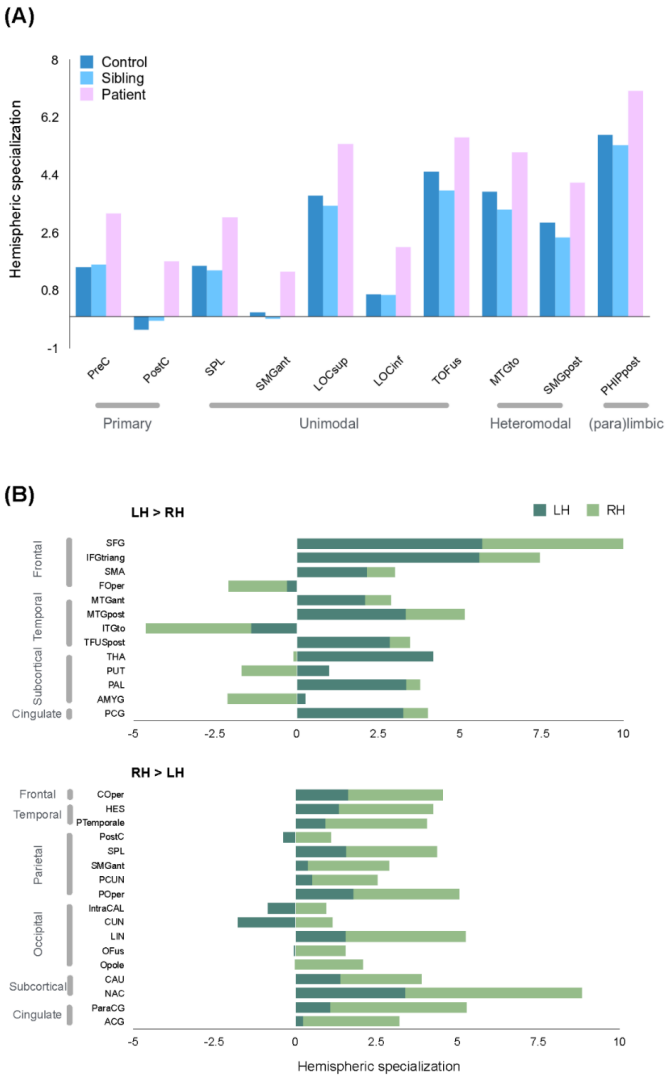

Figure 4 Regional hemispheric specialization showing significant (A) group differences; (B) hemispheric effects ($p < 0.05/56$, Bonferroni correction). For group effects (A), patients exhibited increased hemispheric specialization in unimodal and heteromodal association cortices, sensorimotor cortices and limbic areas. The hemispheric effects (B) indicate higher specialization in frontal and temporal regions and subcortical structures in the left hemisphere, whereas higher specialization was observed in the right hemisphere for parietal, occipital, temporal and subcortical regions. For illustration purpose, regional hemispheric specialization values were averaged over the two hemispheres (A) and over the

three groups (B). Brain regional abbreviation and classification please refer to Supplementary Table 2.

Relationship between interhemispheric connectivity and hemispheric specialization

Comparing patients to controls, group-differences in regional interhemispheric connectivity correlated with changes of regional hemispheric specialization averaged for the left and right hemisphere (r = -0.54, p < 0.001, Figure 5). Co-variation of group-differences in the two measurements was also significant for the sibling-patient comparison (r = -0.54, p < 0.001), and marginally significant for the control-sibling comparison (r = -0.26, p = 0.053). These correlations indicate that regions showing a larger decrease in interhemispheric connectivity in patients as compared to controls or siblings show higher level of hemispheric specialization, indicating that they are preferentially collaborating with regions within the same hemisphere.

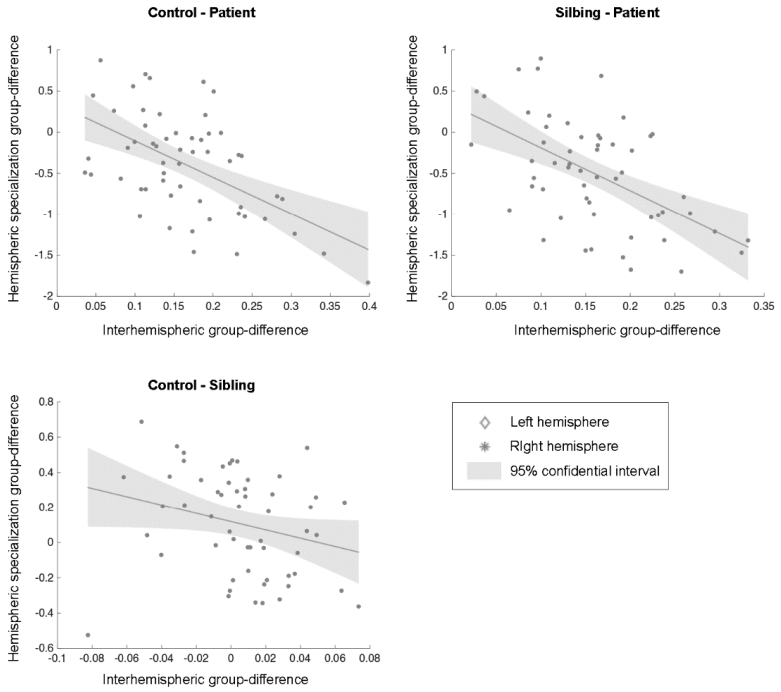

Figure 5 Correlation between group-differences in homotopic interhemispheric connectivity and differences in hemispheric specialization. Decreased regional interhemispheric connectivity is negatively correlated with increased hemispheric specialization in patients as compared to controls (r = -0.54, p < 0.001) and siblings (r = -0.54, p < 0.001). For the control-sibling comparison, co-variation of group-differences in the two connectivity measurements is marginally significant (r = -0.26, p = 0.053).

Correlation between connectivity alterations and clinical factors

Duration of illness was negatively correlated with interhemispheric connectivity (r = -0.26, p = 0.03), and marginally associated with hemispheric specialization (r = 0.24, p = 0.049) in patients (Figure 6). With longer course of illness, patients have more reduced interhemispheric connectivity and a higher level of segregation between the hemispheres. These connectivity alterations are not likely to be attributable to age, as we found no correlations between age and interhemispheric connectivity, or between age and hemispheric specialization in controls and siblings (all p > 0.40). We found no correlations between interhemispheric connectivity or hemispheric specialization with PANSS total scores and medication in patients.

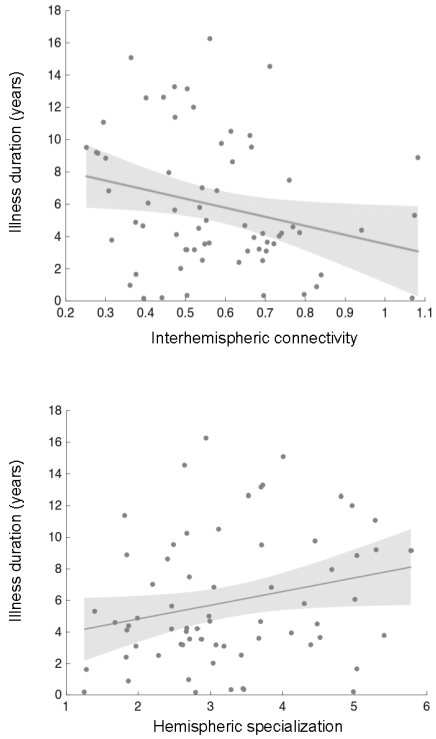

Figure 6 Duration of illness is negatively correlated with global homotopic connectivity (r = -0.26, p = 0.03) and marginally associated with hemispheric specialization (r = 0.24, p = 0.049) in the patient group.

Discussion

The current study examined interhemispheric connectivity and hemispheric specialization in a large cohort of schizophrenia patients, their unaffected siblings, and healthy controls. Compared to controls and siblings, patients showed marked decreases in interhemispheric connectivity, as well as increased hemispheric specialization on a global and regional level. No major deficits in interhemispheric connectivity or hemispheric specialization were found in unaffected siblings of patients. Moreover, decreased interhemispheric connectivity was found to correlate with duration of illness in patients, suggesting a deterioration of interhemispheric information connectivity with ongoing illness. Taken together, our study shows impaired interhemispheric connectivity and a more segregated hemispheric connectivity profile in schizophrenia patients and suggests that these abnormalities are likely to be characteristics of established disease, rather than an expression of preexistent risk for the disorder.

The first question we set out to answer is whether impairments in interhemispheric connectivity are present in patients with schizophrenia and, if so, whether these impairments relate to preexistent familial risk for the illness. To answer this question, we examined a group of unaffected siblings of schizophrenia patients, who share 50% of their genetic information with affected probands on average. In contrast to a previous study reporting a subtle decline of regional interhemispheric connectivity strength in siblings as compared to controls (Guo et al., 2014), our current study does not find significant reductions in interhemispheric connectivity in unaffected siblings, suggesting that interhemispheric connectivity impairments may be an overt clinical phenotype of schizophrenia. Alternatively, interhemispheric connectivity impairments may be present from a young age in patients and siblings, but normalize with development in unaffected siblings due to a compensatory mechanism, as has been shown for siblings of childhood-onset schizophrenia patients (Moran et al., 2013; Ordóñez et al., 2016; Zalesky et al., 2015).

Another interest of the current study was to examine disturbances of interhemispheric connectivity in relation to putative abnormalities in hemispheric specialization. We find that schizophrenia patients exhibit increased hemispheric specialization in several unimodal and heteromodal association regions, as well as sensorimotor cortex and limbic areas (Figure 4A), indicating an excessive reduction in cross-hemispheric connectivity relative to within-hemispheric connectivity in these brain regions. The unimodal and heteromodal association cortices have reciprocal connectivity with lower level brain regions, and are involved in the integration of sensory information from multiple modalities (Mesulam, 1998). Previous studies have shown that the cortical network of multimodal brain regions is disproportionately affected in schizophrenia (Bassett et al., 2008; Pearlson et al., 1996; van den Heuvel et al., 2013) and abnormalities in association cortices such as reduced cortical

thickness and fiber integrity have been found in schizophrenia patients (Cannon et al., 2002; Sprooten et al., 2013; Zalesky et al., 2011). Extending previous findings, we suggest that unimodal and heteromodal areas more preferentially connected to ipsilateral brain areas in schizophrenia, at the expense of facilitating cross-hemispheric interactions with a broader range of brain regions.

In terms of the relationship between interhemispheric connectivity and hemispheric specialization, we find a significant correlation between decreased interhemispheric connectivity and increased hemispheric specialization in patients as compared to both controls and siblings (Figure 5). The possible mechanism of co-variation between interhemispheric communication and hemispheric specialization has been postulated in previous studies, which suggest that the emergence of short-distance, hemispherically specialized processing may follow from the constraint of interhemispheric conduction delays (Aboitiz et al., 2003; Hervé et al., 2013; Ringo et al., 1994). For cognitive functions requiring high temporal precision such as language, hemispherically specialized processing may be preferable over bilateral processing (Tzourio-Mazoyer et al., 2010). In line with this hypothesis, a recent study reported a negative correlation between the level of homotopic interhemispheric connectivity and lateralization in healthy children (Gracia-Tabuenca et al., 2018). Based on previous studies and our current findings, we suggest that hemispheric specialization abnormalities in schizophrenia patients are at least partially attributable to interhemispheric disconnectivity.

A strong main effect of hemisphere was found for hemispheric autonomy of many cortical and subcortical regions (Figure 4B). Higher hemispheric specialization in the left hemisphere was found predominately in language-related regions, as well as subcortical regions and posterior cingulate cortex. Regions with higher autonomy in the right hemisphere mainly included parietal and occipital areas, as well as a few frontal, temporal, cingulate, and subcortical regions. This lateralization pattern is consistent with previous findings of a dissociation between left hemisphere specialization in language areas and a right hemisphere dominance in visuospatial and attentional areas (Cai et al., 2013; Gotts et al., 2013; Wang et al., 2014). Interestingly, we found that the hemispheric specialization of Heschl's gyrus and the temporal plane is lower in the left than the right hemispheric regions, indicating that in these primary auditory cortices, left hemispheric regions are more bilaterally connected (low hemispheric specialization), whereas right-sided regions are more ipsilaterally connected (high hemispheric specialization). This connectivity profile may indicate a more important role of the left primary auditory cortex in integration and processing of auditory information (Tervaniemi and Hugdahl, 2003). Connectivity of the caudate nucleus tends to be more ipsilateral in the right hemisphere as compared to the left hemisphere, which is different from the results of a previous study (Mueller et al., 2015), but

is consistent with a right-over-left dopamine receptor availability in this region in healthy subjects (Laakso, 2000; Vernaleken et al., 2007). Other subcortical regions such as thalamus, putamen, pallidum, and amygdala were all found to exhibit higher hemispheric autonomy for the right hemisphere, in line with previous findings of a more bilaterally connected pattern in the right hemisphere (Gotts et al., 2013; Iturria-Medina et al., 2011; Zhong et al., 2017).

Several points need to be taken into consideration when interpreting our results. First, we preformed region-based rather than voxel-based analyses of interhemispheric connectivity and hemispheric specialization. Hence, our definition of hemispheric specialization is different from a previous study based on voxel-wise measurements (Mueller et al., 2015). Region-based and voxel-based approaches each have their benefits and limitations (Hayasaka and Laurienti, 2010; Joliot et al., 2015). We adopted a region-based approach for better sensitivity and interpretability of the results, and to allow us to correlate measurements of interhemispheric connectivity and hemispheric specialization across brain regions. Second, the proportion of male and female subjects are not balanced across groups. To address this issue, we included sex as a covariate when performing group-comparisons. Previous studies have produced mixed results in terms of the effects of sex on interhemispheric connectivity strength (Gracia-Tabuenca et al., 2018; Guo et al., 2014; Zuo et al., 2010). Future studies with a balanced sex distribution would be better suited to examine potential sex differences on interhemispheric connectivity and hemispheric specialization in schizophrenia. Third, we did not find a significant correlation between regional interhemispheric disconnectivity and symptom severity in patients as some previous studies have reported (Hoptman et al., 2012; Li et al., 2015). A possible reason may be that the patients recruited in this study were relatively stable at the time of participation (PANSS total scores 48.38 ± 14.89, see Table 1). Therefore, we cannot rule out the possibility that an association exists between disconnectivity and symptoms in patients with more pronounced clinical symptoms.

Conclusion

This study explored interhemispheric connectivity and hemispheric specialization in a large cohort of schizophrenia patients, their unaffected siblings and healthy controls. Our results indicate that patients exhibit decreased interhemispheric connectivity as compared to both healthy controls and unaffected siblings, and these impairments were found to correlate with increases in the level of hemispheric specialization. These connectivity alterations appear to be primarily related to illness manifestation, as siblings did not show significant changes compared to controls, and connectivity changes in patients correlated with duration of illness. In summary, the current study suggests that decreased interhemispheric connectivity

and associated abnormalities in hemispheric specialization are features of established schizophrenia rather than an expression of preexistent risk for the disorder.

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Supplementary material of

Interhemispheric connectivity and hemispheric specialization in schizophrenia patients and their unaffected siblings

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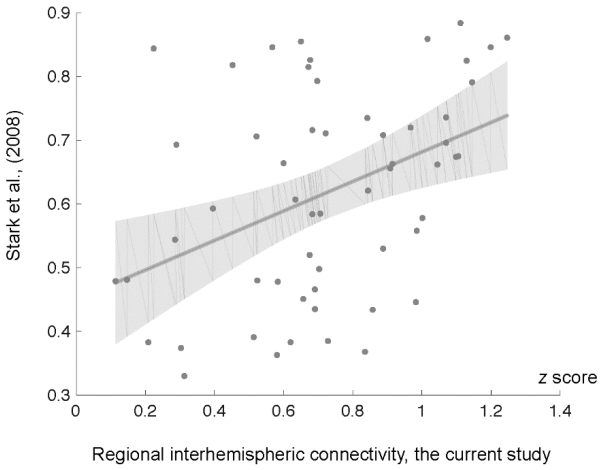
Supplementary Figure 1 Correlation of regional homotopic connectivity

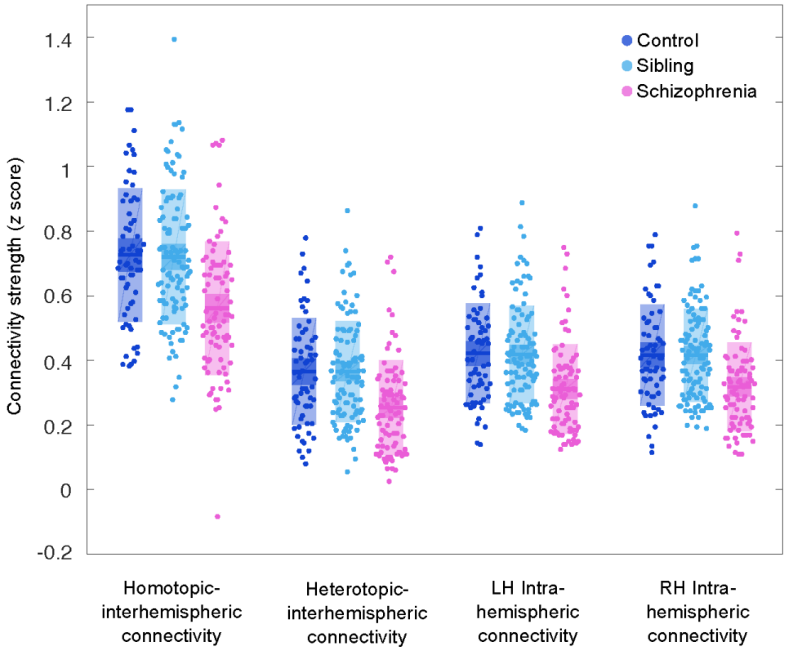
Supplementary Figure 2 Group-comparison of inter- and intra-hemispheric connectivity

Supplementary Table 1 Head motion of participants

Supplementary Figure 2 Regions of Harvard-Oxford atlas



Supplementary Figure 1 Correlation of regional homotopic connectivity strength between the current study and those reported in a previous study (Stark et al., 2008). A significant association was observed (r = 0.41, p = 0.002) despite different MRI processing and analysis methods.



Supplementary Figure 2 Group-comparison of inter- and intra-hemispheric connectivity strength in schizophrenia patients, unaffected siblings and healthy controls. Patients have a pronounced global connectivity decrease ($p < 0.001$). After controlling for this global effect, only homotopic interhemispheric connectivity showed a significant group-effect ($F = 4.31$, $p = 0.01$).

Supplementary Table 1 Head motion of participants

	Controls	Siblings	Patients	*p* value [a]
Motion (before scrubbing)	0.14 (0.04)	0.15 (0.06)	0.15 (0.08)	0.04
Motion (after scrubbing)	0.13 (0.03)	0.14 (0.05)	0.15 (0.05)	0.13
Scrubbed scans [b]	12 (34)	16 (37)	18 (54)	0.17

[a] Head motion and the number of scrubbed scans do not follow normal distribution, thus group median and inter-quartile range were reported. Group-comparisons were performed with Kruskal-Wallis tests.

[b] MR images with frame-wise displacement (FD) > 0.5mm, and 1 scan backward, 2 scans forward were scrubbed.

Supplementary Table 2 Regions of Harvard-Oxford atlas

Region	Abbreviation	Lobe	Hierarchy*
Frontal Pole	Fpole	Frontal	Heteromodal
Insular Cortex	IC	Frontal	Paralimbic
Superior Frontal Gyrus	SFG	Frontal	Heteromodal
Middle Frontal Gyrus	MFG	Frontal	Heteromodal
Inferior Frontal Gyrus, pars triangularis	IFGtriang	Frontal	Heteromodal
Inferior Frontal Gyrus, pars opercularis	IFGoper	Frontal	Heteromodal
Precentral Gyrus	PreC	Frontal	Primary
Temporal Pole	Tpole	Temporal	Paralimbic
Superior Temporal Gyrus, anterior	STGant	Temporal	Unimodal
Superior Temporal Gyrus, posterior	STGpost.	Temporal	Unimodal
Middle Temporal Gyrus, anterior	MTGant	Temporal	Heteromodal
Middle Temporal Gyrus, posterior	MTGpost	Temporal	Heteromodal
Middle Temporal Gyrus, temporooccipital part	MTGto	Temporal	Heteromodal
Inferior Temporal Gyrus, anterior	ITGant	Temporal	Unimodal
Inferior Temporal Gyrus, posterior	ITGpost	Temporal	Unimodal
Inferior Temporal Gyrus, temporooccipital part	ITGto	Temporal	Unimodal
Postcentral Gyrus	PostC	Parietal	Primary
Superior Parietal Lobule	SPL	Parietal	Unimodal
Supramarginal Gyrus, anterior	SMGant	Parietal	Unimodal
Supramarginal Gyrus, posterior	SMGpost	Parietal	Heteromodal
Angular Gyrus	ANG	Parietal	Heteromodal
Lateral Occipital Cortex, superior	LOCsup	Occipital	Unimodal
Lateral Occipital Cortex, inferior	LOCinf	Occipital	Unimodal
Intracalcarine Cortex	IntraCAL	Occipital	Primary
Frontal Medial Cortex	FMC	Frontal	Paralimbic
Supplementary Motor Cortex	SMA	Frontal	Unimodal
Subcallosal Cortex	Subcallosal	Frontal	Paralimbic
Paracingulate Gyrus	ParaCG	Cingulate	Heteromodal

Region	Abbreviation	Lobe	Hierarchy*
Cingulate Gyrus, anterior	ACG	Cingulate	Paralimbic
Cingulate Gyrus, posterior	PCG	Cingulate	Paralimbic
Precuneous Cortex	PCUN	Parietal	Heteromodal
Cuneal Cortex	CUN	Occipital	Unimodal
Frontal Orbital Cortex	FOrb	Frontal	Paralimbic
Parahippocampal Gyrus, anterior	PHIPant	Temporal	Paralimbic
Parahippocampal Gyrus, posterior	PHIPpost	Temporal	Paralimbic
Lingual Gyrus	LIN	Occipital	Unimodal
Temporal Fusiform Cortex, anterior	TFUSant	Temporal	Unimodal
Temporal Fusiform Cortex, posterior	TFUSpost	Temporal	Unimodal
Temporal Occipital Fusiform Cortex	TOFus	Temporal	Unimodal
Occipital Fusiform Gyrus	OFus	Occipital	Unimodal
Frontal Operculum Cortex	FOper	Frontal	Unimodal
Central Opercular Cortex	COper	Frontal	Unimodal
Parietal Operculum Cortex	POper	Parietal	Unimodal
Planum Polare	PPolare	Temporal	Unimodal
Heschls Gyrus (includes H1 and H2)	HES	Temporal	Primary
Planum Temporale	PTemporale	Temporal	Unimodal
Supracalcarine Cortex	SupraCAL	Occipital	Unimodal
Occipital Pole	Opole	Occipital	Primary
Brain-Stem	BS	Subcortical	Subcortical
Thalamus	THA	Subcortical	Subcortical
Caudate	PUT	Subcortical	Subcortical
Putamen	CAU	Subcortical	Subcortical
Pallidum	PAL	Subcortical	Subcortical
Hippocampus	HIP	Subcortical	Limbic
Amygdala	AMYG	Subcortical	Limbic
Accumbens	NAC	Subcortical	Subcortical

* Functional hierarchy division based on study of Mesulam (1998) and Stark et al., (2008).

Diffusion MRI derived free-water imaging measures in schizophrenia patients and their non-psychotic siblings

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In submission

Abstract

Free-water imaging is a newly proposed diffusion magnetic resonance imaging (dMRI) processing technique, which separately models water diffusion that is hindered by fiber tissue and water freely disperse in the extracellular space. Using this technique, studies have shown that schizophrenia patients are characterized by a lower level of fiber integrity and a higher free-water fractional volume. It is unknown, however, whether such abnormalities are expressions of preexisting (genetic) risk for schizophrenia or a direct manifestation of the illness. To investigate the contribution of familial risk to white matter abnormalities, we used linear mixed modelling to analyze free-water fraction (FW) and fractional anisotropy of the tissue compartment (FA_t) in a large cohort of 471 subjects including 161 schizophrenia patients, 182 of their non-psychotic siblings, and 128 healthy controls. Patients did not show significant group-differences on FA_t as compared to controls, but exhibited a higher FW relative to controls and siblings in the left hemisphere uncinate fasciculus, superior corona radiata and fornix / stria terminalis. We also found that FW accumulation in patients is related to, but not solely explained by, ventricle enlargement. Siblings did not show significant FW abnormalities. However, they had an overall higher level of FA_t as compared to controls and patients, in agreement with previous findings in the same cohort. Taken together, the results are suggestive of compensatory changes of FA_t in siblings and indicate that extracellular free-water accumulation in patients is likely to be a disease-related trait characteristic rather than an expression of familial risk for schizophrenia.

Keywords

diffusion-weighted imaging; free-water imaging; schizophrenia; siblings; familiality

Introduction

Schizophrenia is a disabling neuropsychiatric disorder (Saha et al., 2005; Whiteford et al., 2013) with an estimated heritability of 67 ~ 81% (Hilker et al., 2018; Sullivan et al., 2003; Voineskos, 2015). The cause of schizophrenia is not yet known but it is hypothesized that aberrant communication between brain regions plays a key role in the etiology of the disease (Stephan et al., 2009). The connections between distributed gray matter regions are formed by white matter fiber bundles that allow fast and efficient transfer of neural information. Diffusion-weighted magnetic resonance imaging (dMRI) allows us to non-invasively study various characteristics of white matter fiber bundles based on the diffusion of water molecules along these fibers (Johansen-Berg and Rushworth, 2009; Tournier et al., 2011). One widely used dMRI analysis method, diffusion tensor imaging (DTI), applies a tensor model to describe water diffusion profiles (Basser, 1995; Basser et al., 1994). From the fitted tensor, measurements such as fractional anisotropy (FA) can be computed, which often serve as an index of white matter fiber integrity.

Previous DTI studies have reported lower FA in schizophrenia patients in various white matter tracts including association fibers: the arcuate and uncinate fasciculus (de Weijer et al., 2011), fronto-occipital fasciculus (Kubicki et al., 2005; Lee et al., 2013), superior and inferior longitudinal fasciculus (Shergill et al., 2007), cingulum bundle (Kubicki et al., 2003; Mori et al., 2007); commissural fibers of the fornix (Zhou et al., 2008) and corpus callosum (Patel et al., 2011; Rotarska-Jagiela et al., 2008) and projection fibers: corona radiata and internal capsule (Kubicki et al., 2005). For reviews and meta-analyses of DTI results in schizophrenia see (Ellison-Wright and Bullmore, 2009; Kelly et al., 2018; Kubicki and Shenton, 2014; Melonakos et al., 2011). Other studies, however, did not find disease-related differences in FA (Clark et al., 2012; Foong et al., 2002; Lee et al., 2012; Mandl et al., 2010). Possible explanations for these varying results include the type of population studied (e.g. chronic versus first-episode patients) (Kong et al., 2011; White et al., 2011) and the non-specific nature of FA alterations in relation to biological properties of white matter fibers such as myelination, axonal alignment, axon density, and the influence of mixed types of tissue in an imaging voxel (Alexander et al., 2001; Beaulieu, 2002; Jones, 2010).

Among efforts to improve the accuracy of DTI to model fiber tissue (Alexander et al., 2001; Hirsch et al., 1999; Pierpaoli and Jones, 2004), the dMRI derived free-water imaging approach (Pasternak et al., 2009) introduced a bi-tensor model to separately model water molecules that are in the proximity of white matter fibers and water molecules that are free to diffuse in the extracellular space. The anisotropy of water diffusion in the proximity of white matter (quantified by tissue-FA or FA_t) is considered a better proxy for microstructural tissue related changes than FA calculated from the single-tensor model (Bergamino et al., 2017).

The free-water fraction (FW) is sensitive to processes that affect the extracellular space, such as neuroinflammation and atrophy (Pasternak et al., 2018). This free-water imaging technique can be applied to conventional DTI acquisitions, increasing the specificity of tissue modelling (Bergamino et al., 2017) and improving test-retest reproducibility (Albi et al., 2017). Previous free-water imaging studies of schizophrenia have reported a higher FW in patients, particularly in the early stage of the disease (Lyall et al., 2018; Pasternak et al., 2012), and a lower level of FA_t (Oestreich et al., 2017; Pasternak et al., 2015). However, the question is whether these white matter abnormalities reflect processes that are part of the pathophysiological pathway leading up to schizophrenia or are rather secondary effects of the disease. A possible strategy to address this issue is to examine the contribution of familial (possibly reflecting genetic) risk for schizophrenia to white matter abnormalities.

Studies of healthy populations indicate that FA measurements are heritable (Blokland et al., 2012; Brouwer et al., 2010b; Chiang et al., 2011; Kochunov et al., 2015) and a twin study has shown that genetic variants influencing FA overlap with risk variants for schizophrenia (Bohlken et al., 2016). However, studies investigating FA in non-psychotic siblings of schizophrenia patients have shown varying results. Some studies reported limited FA decreases in relatives as compared to controls (Camchong et al., 2009; Skudlarski et al., 2013) while others did not find significant FA differences between relatives and controls (Harms et al., 2015). A study by our group on the cohort described here actually reported higher FA in non-psychotic siblings relative to controls in the left and right arcuate fasciculus (Boos et al., 2013). The question is if these varying results may (in part) be due to the non-specific nature of conventional FA measurements. If so, separately analyzing FA_t and FW may increase the sensitivity to detect abnormalities in unaffected relatives.

In the current study, we calculate FA_t and FW using the free-water imaging method in a large dataset of 471 subjects including schizophrenia patients, their non-psychotic siblings and healthy controls (Boos et al., 2013).We aim to examine how extracellular free-water accumulation and impairments in fiber anisotropy in patients with schizophrenia relate to familial risk for the disorder. To obtain more insight into the potential influence of specific analysis methodologies, we apply three analyses that are sensitive to effects at different resolutions: a global white matter skeleton analysis; an atlas-based region of interest (ROI) analysis; and a voxel-based analysis. As a previous study suggested that FW may correlate with ventricle size (Pasternak et al., 2018), a secondary analysis is performed to calculate the correlation between ventricle size and diffusion measurements and to assess the influence of ventricle size on the group-comparison of diffusion measurements.

Methods

Subjects

A total of 483 subjects were recruited at the University Medical Center Utrecht (UMCU). Twelve subjects were excluded due to poor image quality (2 controls, 7 siblings, 3 patients), leaving data from 471 subjects used for further analysis, including 161 schizophrenia patients, 182 non-psychotic siblings of schizophrenia patients, and 128 healthy controls. This dataset has been reported in a previous DTI study of our group using fiber-based analyses based on single-tensor modelling (Boos et al., 2013). The data was collected as part of the Genetic Risk and Outcome of Psychosis (GROUP) study in the Netherlands (Korver et al., 2012). The medical ethics committee (METC) of the UMCU approved the study and all participants provided written informed consent prior to participation.

Inclusion criteria for all subjects included: 1) age between 16 and 50 years; 2) good command of the Dutch language; 3) being able and willing to give written informed consent. Presence or absence of current and lifetime psychopathology was evaluated using the Comprehensive Assessment of Symptoms and History (CASH) interview (Andreasen et al., 1992). Patients were included if they met diagnostic criteria for non-affective psychotic disorder according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association, 2000). Symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). The type and dosage of antipsychotic medication at the time of scanning was recorded and converted to a chlorpromazine equivalent dose (Kroken et al., 2009).

We recruited 182 non-psychotic siblings including 102 patient-sibling pairs or triplets. A family identification code (family ID) was assigned to each participant to keep track of their pedigrees. Healthy controls were selected through a system of random mailings. Exclusion criterion for siblings and controls included any current or previous diagnosis of a psychotic disorder. Furthermore, controls were excluded if a lifetime psychotic disorder was diagnosed in a first- or second-degree family member, as assessed by the Family Interview for Genetic Studies (Maxwell, 1992). Demographics and clinical information are described in Table 1.

Table 1 Demographic and clinical characteristics of 471 subjects (128 controls, 182 siblings, 161 patients)

	Controls	Siblings	Patients	*p* value [a]
Demographic information				
Age	27.27 ± 8.76	27.36 ± 6.85	26.57 ± 5.91	0.55
Sex (male/female)	62 / 66	82 / 100	34 / 127	<0.001
IQ	111.52 ± 14.81	101.84 ± 14.19	93.51 ± 15.57	<0.001
Handedness (Right/Left/Mix)	113 / 12 / 2	161 /10 / 9	142 / 8 / 5	0.29
Scanning factors				
Motion (RMS) [b]	0.23 (0.18, 0.30)	0.25 (0.18, 0.31)	0.25 (0.19, 0.34)	0.38
Scanner	79 / 49	102 / 80	79 / 82	0.14
Clinical characteristics				
PANSS total	--	--	61.39±17.05	--
PANSS positive	--	--	15.02±5.55	--
PANSS negative	--	--	15.20±5.50	--
PANSS general psychopathology	--	--	31.16±8.64	--
Duration (years) [b]	--	--	4 (2, 6)	--
Antipsychotics (chlorpromazine equivalent dosage, mg/day) [b]	--	--	400 (300, 600)	--

[a] Group comparison of age and IQ were conducted using one-way ANOVA. Nominal variables (sex, handedness, scanner) were compared with chi-square statistics.

[b] Head motion, illness duration and dosage of antipsychotic medication do not follow a normal distribution, therefore median (25th, 75th percentile) values are reported. Group comparison of head motion was performed with Kruskal-Wallis statistics.

MRI data acquisition

MRI scans were acquired on two 1.5T Philips Achieva Scanners with the same set of scanning parameters. For each subject, a three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head was acquired (256 × 256 matrix, TE = 4.6 ms, TR = 30 ms, flip angle = 30°, 160–180 contiguous slices; 1 × 1 × 1.2 mm^3 voxels, FOV = 256 mm/70%). Diffusion data were collected using a single-shot echo planar imaging sequence (EPI). Two sets of DTI scans (32 diffusion-weighted volumes with different non-collinear diffusion directions with b-factor = 1000 s/mm^2 and 4 diffusion-unweighted volumes with b-factor = 0 s/mm^2) were acquired with the following parameters: parallel imaging SENSE factor = 2.5; flip angle = 90°; 60 slices of 2.5 mm isotropic voxels without slice gap; acquisition matrix = 96 × 96; reconstruction matrix = 128 × 128; FOV = 240 mm; TE = 88 ms; TR = 9822 ms.

MRI processing

Preprocessing of the DTI images was done using a combination of MRtrix (http://www.mrtrix.org) (noise filtering) and FSL (http://www.fmrib.ox.ac.uk/fsl/). Root mean square (RMS) movement distance was calculated from translational and rotational parameters obtained in the registration step and stored to be used as a covariate in further statistical analysis (Taylor et al., 2016). Computation of FW and FA_t values was performed using software described in Pasternak et al., (2009). Tract based spatial statistics (TBSS) was used to create a white matter skeleton for each subject, yielding a point-by-point correspondence across all subjects allowing group comparisons.

Global analysis

For every subject global FA_t and FW values were computed over the intersection between the skeleton voxels and all 48 regions of interest (ROIs) defined in the JHU ICBM-DTI-81 atlas (Mori et al., 2008), and entered into linear mixed modeling as dependent variable (*Y*) for statistical comparisons:

$$Y = Group + Age + Sex + Motion + Scanner + GroupxAge + (1|FamilyID) + \varepsilon$$

Fixed effects included group (patients/siblings/controls), age, sex, head motion (RMS), scanner, an interaction term group × age, and a random effect that modeled pedigree relationships of subjects.

The model was first tested with a potential interaction effect (group × age). If not significant, the interaction term was dropped and the reduced model was fitted. The full model had 471 observations and 8 coefficients, leaving 462 residual degrees of freedom.

The reduced model (without interaction effect) had 6 coefficients and 464 residual degrees of freedom. The main effect of group and post-hoc bivariate comparisons were reported for global FA_t and FW.

ROI analysis

To obtain more detailed information on which brain regions are involved, we analysed each of the 48 ROIs separately. For each ROI, a linear mixed model analysis was performed in the same manner as for the global measurements, controlling for multiple comparisons using a false discovery rate (FDR) of $q < 0.05$.

Voxel-based analysis

Voxel-based analysis provides better spatial resolution to localize possible FA_t and FW abnormalities as compared to global and ROI-based analyses. Voxel-wise FA_t and FW values were compared between each pair of subject groups using the *randomise* function from the FSL package (Winkler et al., 2014). Age, sex, motion and scanner were included as covariates. Statistics of the voxel-wise analysis were calculated using the threshold-free cluster enhancement (TFCE) approach (Smith and Nichols, 2009) and permutation analysis (5000 iterations). Multiple comparisons were corrected using a family-wise error (FWE) rate of 0.05.

Secondary analysis of ventricle volume influence diffusion measurements

The free-water imaging technique was originally developed to correct FA for extracellular free-water signal contamination. It has been suggested that FW may be related to ventricle size (Pasternak et al., 2018). To determine potential influence of ventricle volume on diffusion measurements, we first tested associations between ventricle volume and diffusion measurements, and then included ventricle volume as an additional fixed factor in our linear mixed models.

To obtain a measure of ventricle volume, T1-weighted images were normalized to Talairach space, corrected for field inhomogeneities. The intracranial volume was segmented into gray matter, white matter and ventricles (Brouwer et al., 2010a), including the lateral and third ventricles (Schnack et al., 2001). A detailed description of the processing pipeline can be found in Boos et al., (2013). We first performed correlation analyses between global FA_t and FW (corrected for age, sex, scanner and head motion) and ventricle volume (corrected for age, sex and scanner) in the three subject groups separately. We also examined correlations between ventricle volume and averaged FA_t and FW values for the 48 ROIs ($q < 0.05$, FDR corrected for 48 × 3 comparisons). Then, for diffusion measurements with significant group-differences, we included ventricle volume as an

additional fixed effect in linear regression models to determine if group effects remained significant after correcting for ventricle size.

Correlations between DTI measurements and clinical measures

To examine white matter fiber changes in relation to markers of illness severity, we performed correlation analyses between global FA_t and FW and clinical metrics including duration of illness, PANSS total and subscale scores ($p < 0.05$, uncorrected). To examine the influence of antipsychotic medication on white matter diffusion properties, correlation coefficients were calculated between global FA_t, FW and chlorpromazine equivalent dosage of medication. DTI measurements were corrected for age, sex, scanner and head motion before entering correlation analysis.

Results

Global analyses

Linear mixed model analysis revealed no significant effects for the interaction term group × age on FA_t ($F = 1.48$, $p = 0.23$) or FW ($F = 0.98$, $p = 0.38$), which was therefore dropped. The reduced linear mixed model analysis showed that there was a significant main effect of group for global FA_t ($F = 4.30$, $p = 0.01$, Figure 1). Post-hoc bivariate comparisons showed that global FA_t was higher in siblings as compared to both patients ($t = 2.69$, $p = 0.01$) and controls ($t = 2.13$, $p = 0.03$), while no difference was found between patients and controls ($t = 0.23$, $p = 0.82$). The main effect of group on global FW was not significant ($F = 1.98$, $p = 0.14$), and statistics of two-group comparisons are $t = 1.55, 1.81, 0.09$, $p = 0.12, 0.07, 0.93$ for patients vs controls, patients vs siblings and siblings vs controls, respectively.

We found a significant main effect of age on both global FA_t ($F = 49.67$, $p < 0.001$) and FW ($F = 6.88$, $p = 0.01$, Supplementary Figure 1). The main effect of sex was not significant for global FA_t ($F = 2.35$, $p = 0.13$) and marginally significant for global FW ($F = 3.77$, $p = 0.05$).

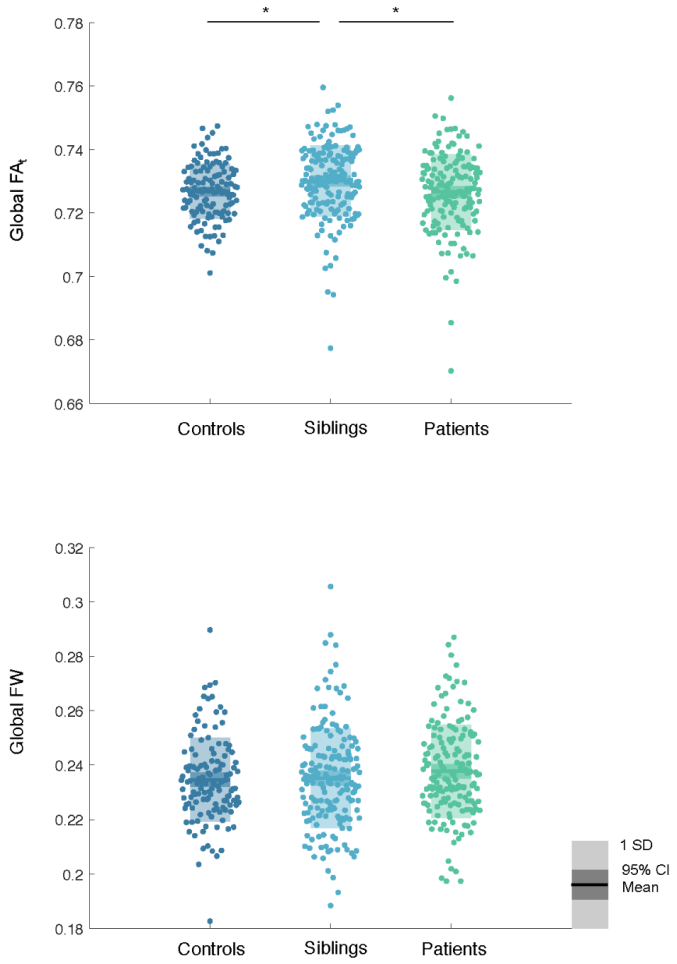

Figure 1 Group comparisons of global FA_t and FW. Main effect of group is significant for global FA_t (F = 4.30, p = 0.01), which was higher in siblings as compared to both patients (t = 2.69, p = 0.01) and controls (t =2.13, p = 0.03). Group effect for global FW was not significant (F = 1.98, p = 0.14).

ROI analyses

For FA_t, the ROI-based (intermediate level) mixed model analysis did not reveal significant group × age interaction effects and consequently the interaction term was dropped in further analyses. Main effects of group on FA_t were not significant in the reduced model after multiple comparison correction (Supplementary Table 1). For FW, the interaction effect of group × age was only significant for the left cerebral peduncle (F = 7.70, p = 0.001, FDR corrected, q < 0.05). In the reduced model, group effects of ROI averaged FW were

significant after FDR correction in three left hemisphere ROIs: uncinate fasciculus (F = 5.95, p = 0.003), superior corona radiata (F = 6.05, p = 0.003), and fornix / stria terminalis (F = 12.72, p < 0.001, Figure 2). Post-hoc analyses showed that patients had higher FW values for these ROIs compared to both controls and siblings (p < 0.05, Supplementary Table 1).

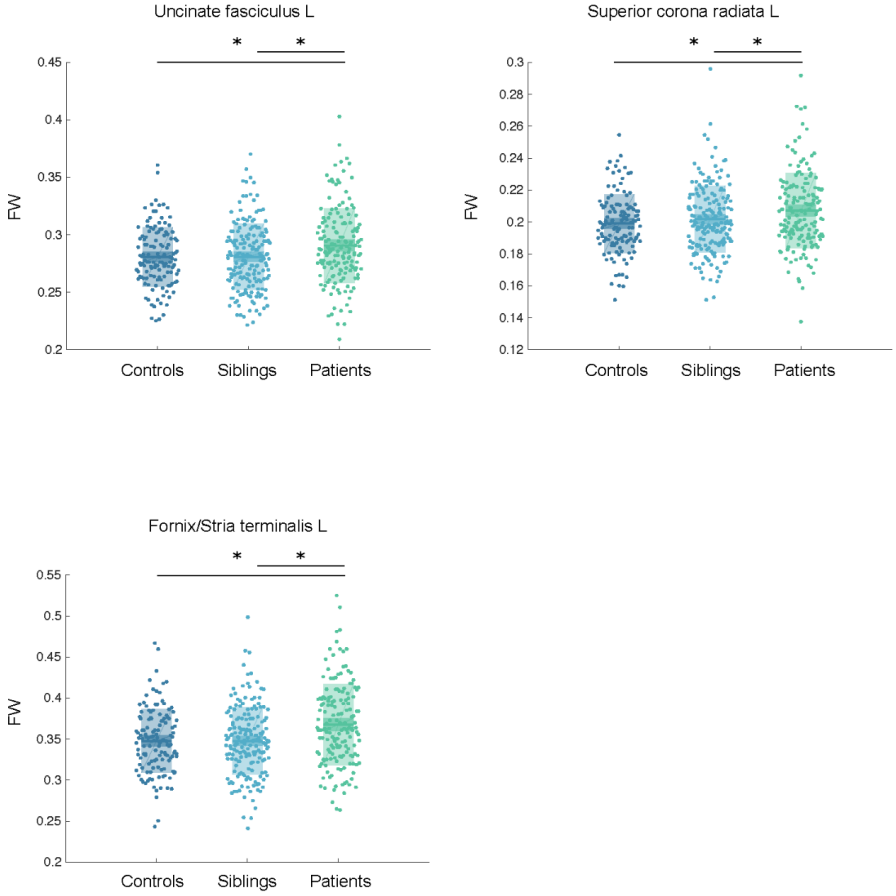

Figure 2 Main effects of group were significant for fiber averaged FW in the left uncinate fasciculus, superior corona radiata and fornix / stria terminalis (FDR corrected, q < 0.05). Post-hoc analysis showed that patients have increased level of FW as compared to both controls and siblings (all p < 0.05, Supplementary Table 1).

Voxel-based analyses

Voxel-wise comparisons showed higher FA_t values in siblings compared to patients in the corpus callosum, bilateral internal capsule, corona radiata, thalamic radiation, superior longitudinal fasciculus and cerebral peduncle (FWE corrected, p < 0.05, Figure 3). Voxel-

wise FA_t values in controls did not differ from either patients or siblings. Group-comparisons of voxel-based FW showed no significant differences between the subject groups.

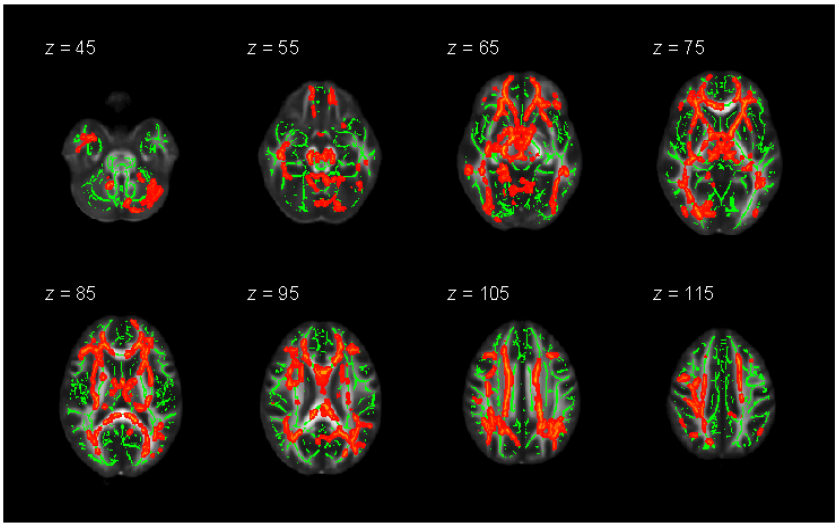

Figure 3 Voxel-based analysis showed significant clusters (in red-yellow, thickened for visualization purpose) in group comparison of FA_t between schizophrenia patients and their non-psychotic siblings (FWE corrected, $p < 0.05$).

Secondary analysis of ventricle volume influence diffusion measurements

We found significant associations between global FW and ventricle volume in all three subject groups (controls: $r = 0.21$, $p = 0.02$; siblings: $r = 0.16$, $p = 0.03$; patients: $r = 0.21$, $p = 0.01$, Supplementary Figure 2), whereas correlations between global FA_t and ventricle volume were not significant (controls: $r = 0.02$, $p = 0.77$; siblings: $r = -0.09$, $p = 0.26$; patients: $r = 0.09$, $p = 0.24$). In addition, ventricle volume is significantly correlated with ROI averaged diffusion measurements for fibers nearby ventricles (corpus callosum, fornix, fornix / stria terminalis), and with ROI averaged FW in the left superior frontal-occipital fasciculus and FA_t in the posterior and retrolenticular part of internal capsule in all three subject groups (Supplementary Table 2).

Including ventricle volume as a fixed factor in the linear regression model did not change the nature of our findings. The main effect of group on global FA_t remained significant ($F = 3.10$, $p = 0.046$), and siblings showed higher global FA_t than patients ($t = 2.30$, $p = 0.02$). Group-differences of FW in the three ROIs identified by the ROI-based analyses (left hemisphere uncinate fasciculus, superior corona radiata radiata and fornix / stria terminalis) remained significant after including ventricle volume as a regressor (Supplementary Table 3).

Correlations with clinical measures

We did not find significant correlations between global FA_t or global FW and PANSS scores or illness duration ($p > 0.05$, uncorrected) in patients. Correlations between antipsychotic medication intake (in chlorpromazine equivalent dosage) and global FW or FA were not significant ($r = -0.05, 0.02$; $p = 0.61, 0.82$ respectively).

Discussion

This study investigated FA_t and FW in a large cohort of schizophrenia patients, their non-psychotic siblings and healthy controls. Compared to controls, patients showed no significant group-difference of FA_t, but exhibited a higher level of FW in three white matter ROIs in the left hemisphere relative to controls and siblings. Siblings did not differ from controls on FW, suggesting that the abnormalities in patients are likely to be disease-related effects rather than a reflection of familial risk for the illness. Global and voxel-wise group-comparisons revealed higher FA_t in siblings relative to patients, consistent with a previous study by our group using fiber-based analysis based on a single tensor model in the same dataset (Boos et al., 2013). Moreover, examining the influence of ventricle volume on diffusion measurements, we found that although global FW was positively correlated with ventricle size, correcting for ventricle size in group-comparisons did not change our main results.

ROI-based analyses showed higher FW values in patients as compared to controls and siblings in three ROIs in the left hemisphere representing the uncinate fasciculus, superior corona radiata, and fornix / stria terminalis (Figure 2). These regions partially overlap with results from a previous study reporting higher FW in chronic patients in the left corona radiata and corpus callosum (Pasternak et al., 2015). The identified regions with higher FW have been implicated in schizophrenia using FA uncorrected for free-water (Kawashima et al., 2009; Kubicki et al., 2005; Lee et al., 2013; Mandl et al., 2015; Mori et al., 2007; Zhou et al., 2008), suggesting that fiber impairments in these bundles could be related to free-water accumulation. Indeed, a recent study performing free-water corrected and uncorrected DTI analyses found that fibers exhibiting reductions in free water-uncorrected FA in patients largely overlap with regions showing FW increases (Lyall et al., 2018). These findings suggest that abnormalities identified by uncorrected FA may in part, relate to extracellular free-water accumulation in patients and that future studies should employ FW correction.

Our study demonstrates that FW abnormalities in patients found in the ROI-based analysis are more likely to be clinical phenotypes of schizophrenia than an expression of preexisting (genetic) risk for the illness, which to the best of our knowledge has not yet been reported. Previous studies suggested that free-water accumulation may change with disease progression, from being widespread in the first-episode (Lyall et al., 2018; Pasternak et al.,

2012) to more confined in the chronic stage (Oestreich et al., 2017; Pasternak et al., 2015). Patients recruited in the current study are not in the acute phase of the disease, but have a relatively short duration of illness of 4 years (median). The extent of identified abnormalities in our sample lies in between those reported in patients with acute illness (Lyall et al., 2018; Pasternak et al., 2012) and patients with chronic schizophrenia (Oestreich et al., 2017; Pasternak et al., 2015).

We found an overall higher level of FA_t in siblings as compared to patients and controls (Figure 1), as well as localized group differences between siblings and patients in voxel-based analyses (Figure 3). In a previous study of our group using conventional single tensor modelling technique on the same dataset, we also found higher uncorrected FA in siblings than patients and controls, which may indicate a compensatory mechanism in siblings who bear an increased familial risk for schizophrenia but are not clinically affected (Boos et al., 2013). Finding no evidence for white matter impairments in siblings is at odds with a previous twin study by our group showing an overlap between schizophrenia liability and FA (Bohlken et al., 2016) and imaging genetic studies implicating schizophrenia risk genes in white matter integrity (Mothersill et al., 2012). Several reasons may explain this discrepancy: First, non-psychotic siblings have a lower genetic burden than discordant monozygotic twins of patients. The shared genetic variation of schizophrenia liability and FA was estimated to be 4.7% in the twin study (Bohlken et al., 2016), which is relatively low and may thus not show up in a sibling cohort. Second, if white matter fiber integrity was ever affected in siblings, such abnormalities may ameliorate or be compensated during development (Mizuno et al., 2016; Moran et al., 2013; Ordóñez et al., 2016). Indeed, longitudinal studies have reported that siblings of childhood-onset schizophrenia patients exhibit grey matter abnormalities in prepubescence, but that these deficits normalize before adulthood (Mattai et al., 2011; Zalesky et al., 2015). In short, we cannot rule out the possibility that genetic factors are involved in FA and FW abnormalities in schizophrenia, but these effects may be too subtle to show up in non-psychotic siblings, or may have normalized by the time of adulthood.

Our secondary analysis revealed a significant association between global FW and ventricle size, which may either be due to methodological confounds or may reflect a shared biological process. Methodologically, signal from ventricle voxels could spill over into white matter skeleton voxels due to misregistration, causing a spurious correlation between FW and ventricle measurements. In our analysis, we calculated global and ROI averaged diffusion measurements based on the intersection of white matter skeleton and the JHU ICBM-DTI-81 atlas (Mori et al., 2008), resulting in a conservative selection of white matter voxels and limiting the chance for spill-over of signal from adjacent ventricle voxels. Currently, we are not aware of studies reporting a shared biological process underlying FW

and ventricle size. Future research using more direct quantifications of extracellular water could focus on studying such a possible biological mechanism (Nicholson and Hrabětová, 2017; Simon and Iliff, 2016).

Several limitations should be considered when interpreting results of the current study. First, the number of females and males participating in this study was not balanced across the groups as there are more males in the patient group as compared to siblings and controls. Considering reported sex differences in regional microstructural properties in previous DTI studies (Hsu et al., 2008; Menzler et al., 2011), this could have influenced our results, even though we corrected for sex in all statistical analyses. Second, we did not find significant associations between clinical characteristics and diffusion measurements in patients, which is inconsistent with a previous study suggesting correlations between FA_t and negative symptoms (Pasternak et al., 2015). The current negative results may be due to our patient sample has a relatively short illness course (median duration of illness is 4 years) and low symptom levels (mean PANSS total score of 61.39). Third, the linear mixed modeling method used in our global level and ROI-based analyses has subtle differences as compared to the linear regression method used in our voxel-based analysis, with the latter method not accounting for familial ties between subjects.

In conclusion, this study in a large cohort of schizophrenia patients, their non-psychotic siblings, and healthy controls finds higher FW in patients as compared to controls and siblings in three white matter tracts in the left hemisphere. These group-differences remained significant after controlling for ventricle size. Siblings did not show significant differences in FW relative to controls, but did exhibit a higher level of FA_t as compared to patients and controls, which may reflect a compensatory mechanism. Our findings suggest that extracellular free-water accumulation in patients is likely to be a clinical manifestation of established disease rather than an expression of preexisting familial risk for schizophrenia.

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Supplementary material of

Diffusion MRI derived free-water imaging measures in schizophrenia patients and their non-psychotic siblings

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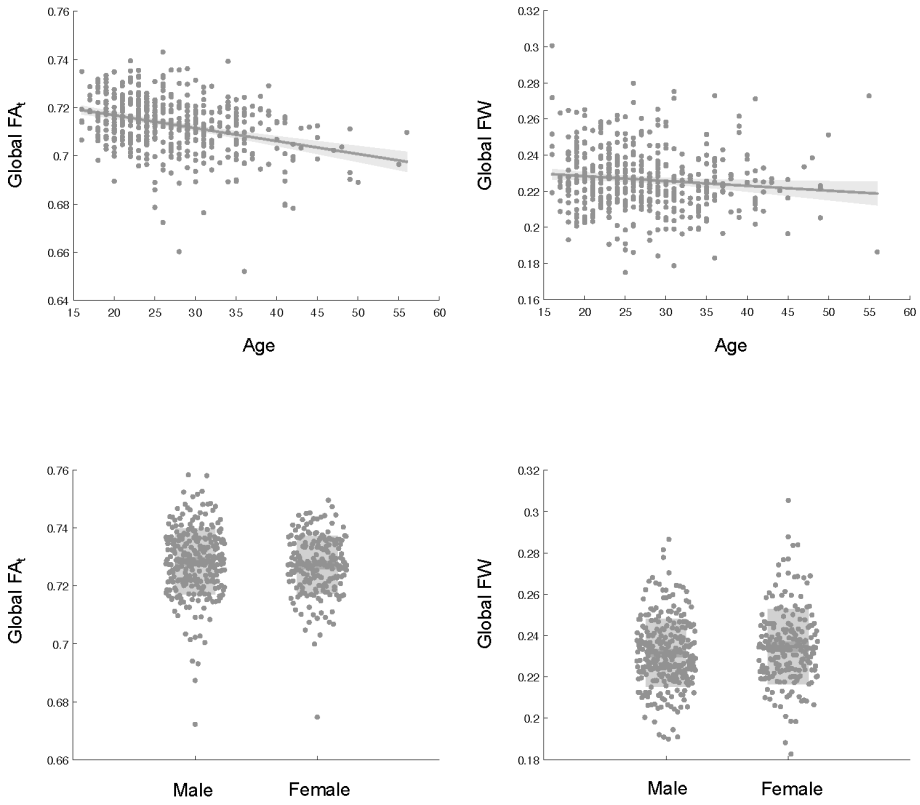
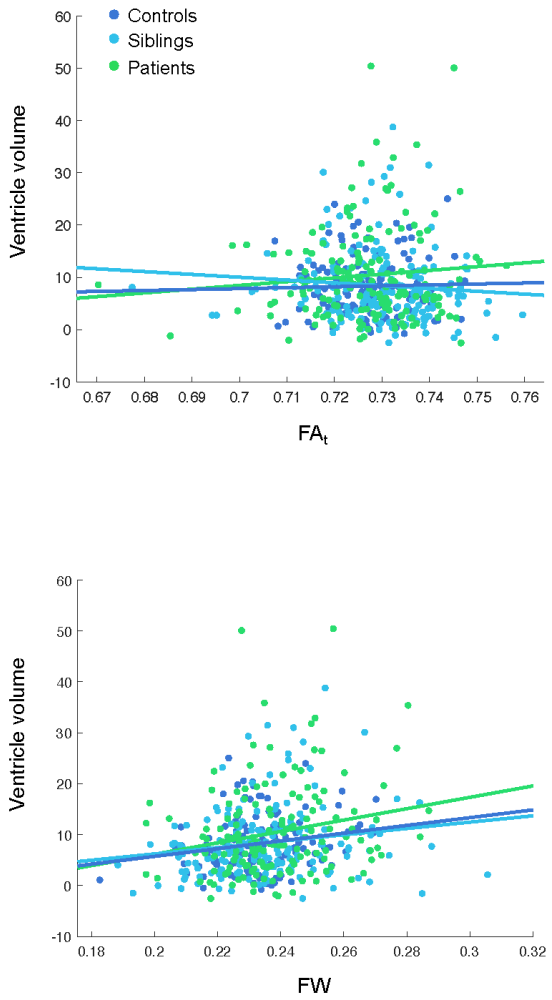
Supplementary Figure 1 Main effect of age and gender on global FA_t and FW
Supplementary Figure 2 Correlation between ventricle volume and global FA_t and FW
Supplementary Table 1 Group main effects and post-hoc comparisons of ROI-based analysis
Supplementary Table 2 Associations between ROI averaged FW / FA_t and ventricle volume
Supplementary Table 3 Secondary analysis including ventricle volume as an additional covariate in group-comparisons of the significant results of the ROI-based analyses



Supplementary Figure 1 Main effect of age was significant for both global FA_t (F = 49.67, p < 0.001) and FW (F = 6.88, p = 0.01). Main effect of sex was not significant on global FA_t (F = 2.35, p = 0.13) and marginally significant for global FW (F = 3.77, p = 0.05).



Supplementary Figure 2 Ventricle volume significantly correlated with global FW in the three subject groups (controls: r = 0.21, p = 0.02; siblings: r = 0.16, p = 0.03; patients: r = 0.21, p = 0.01), but not with global FA_t (controls: r = 0.02, p = 0.77; siblings: r = -0.09, p = 0.26; patients: r = 0.09, p = 0.24).

Supplementary Table 1 Group main effects and post-hoc bivariate comparisons of ROI-based analysis

Tract	Group main effect on FA_t		post-hoc *p* values			Group main effect on FW		post-hoc *p* values		
	F	*p*	Con vs. Sch	Con vs. Sib	Sib vs. Sch	*F*	*p*	Con vs. Sch	Con vs. Sib	Sib vs. Sch
Middle cerebellar peduncle	1.12	0.33	0.23	0.15	0.84	0.10	0.91	0.88	0.67	0.77
Pontine crossing tract	0.10	0.90	0.70	0.95	0.70	0.84	0.43	0.29	0.22	0.89
Genu of corpus callosum	1.50	0.22	0.97	0.18	0.13	0.07	0.93	0.74	0.95	0.75
Body of corpus callosum	5.37	0.00	0.52	0.05	0.00	1.55	0.21	0.09	0.14	0.76
Splenium of corpus callosum	2.36	0.10	0.20	0.66	0.03	0.84	0.43	0.35	0.99	0.23
Fornix	0.68	0.51	0.34	1.00	0.29	3.08	0.05	0.10	0.64	0.02
Corticospinal tract R	0.34	0.71	0.87	0.47	0.52	0.21	0.81	0.67	0.52	0.85
Corticospinal tract L	1.05	0.35	0.23	0.17	0.93	0.44	0.64	0.36	0.48	0.78
Medial lemniscus R	2.01	0.13	0.07	0.07	0.97	1.68	0.19	0.07	0.28	0.38
Medial lemniscus L	1.43	0.24	0.11	0.14	0.84	2.12	0.12	0.20	0.56	0.04
Inferior cerebellar peduncle R	2.82	0.06	0.45	0.21	0.02	0.50	0.61	0.64	0.66	0.32
Inferior cerebellar peduncle L	0.88	0.42	0.77	0.23	0.32	1.56	0.21	0.61	0.26	0.09
Superior cerebellar peduncle R	0.80	0.45	0.49	0.21	0.53	0.06	0.94	0.92	0.75	0.81
Superior cerebellar peduncle L	0.86	0.42	0.27	0.87	0.26	0.42	0.66	0.38	0.75	0.51
Cerebral peduncle R	0.71	0.49	0.32	0.91	0.30	1.67	0.19	0.57	0.29	0.07

Cerebral peduncle L	0.36	0.70	0.61	0.82	0.40	1.76	0.17	0.15	0.92	0.08
Anterior limb of internal capsule R	1.29	0.28	0.18	0.84	0.16	0.03	0.97	0.95	0.82	0.87
Anterior limb of internal capsule L	2.22	0.11	0.26	0.51	0.04	0.98	0.38	0.32	0.88	0.18
Posterior limb of internal capsule R	3.80	0.02	0.01	0.01	0.90	2.53	0.08	0.21	0.46	0.03
Posterior limb of internal capsule L	4.02	0.02	0.01	0.03	0.44	0.54	0.58	0.86	0.46	0.33
Retrolenticular part of internal capsule R	1.66	0.19	0.08	0.46	0.21	0.22	0.80	0.69	0.83	0.51
Retrolenticular part of internal capsule L	0.12	0.89	0.84	0.64	0.77	0.73	0.48	0.56	0.58	0.23
Anterior corona radiata R	5.15	0.01	0.21	0.14	0.00	0.16	0.85	0.75	0.57	0.78
Anterior corona radiata L	5.44	0.00	0.35	0.08	0.00	1.35	0.26	0.43	0.60	0.10
Superior corona radiata R	2.77	0.06	0.51	0.03	0.08	4.98	0.01	0.00	0.38	0.01
Superior corona radiata L	2.12	0.12	0.54	0.06	0.14	6.05	0.00	0.00	0.31	0.01
Posterior corona radiata R	2.06	0.13	0.43	0.05	0.23	1.25	0.29	0.17	0.79	0.19
Posterior corona radiata L	2.20	0.11	0.79	0.07	0.09	2.61	0.07	0.13	0.75	0.03
Posterior thalamic radiation R	1.61	0.20	0.48	0.37	0.08	1.30	0.27	0.65	0.37	0.11
Posterior thalamic radiation L	2.15	0.12	0.52	0.24	0.04	1.90	0.15	0.78	0.19	0.07
Sagittal stratum R	1.61	0.20	0.08	0.35	0.28	0.62	0.54	0.30	0.75	0.40
Sagittal stratum L	0.74	0.48	0.50	0.70	0.23	2.03	0.13	0.27	0.53	0.05

External capsule R	0.87	0.42	0.73	0.49	0.20	0.56	0.57	0.72	0.56	0.30
External capsule L	0.86	0.42	0.47	0.20	0.49	2.41	0.09	0.09	1.00	0.04
Cingulum R	0.26	0.77	0.73	0.79	0.47	0.12	0.89	0.81	0.84	0.63
Cingulum L	0.23	0.80	0.71	0.86	0.50	0.40	0.67	0.98	0.46	0.44
Cingulum (hippocampus) R	0.68	0.51	0.87	0.43	0.27	0.01	0.99	0.92	0.88	0.96
Cingulum (hippocampus) L	0.56	0.57	0.33	0.35	0.93	2.49	0.08	0.05	0.83	0.05
Fornix / Stria terminalis R	0.31	0.73	0.88	0.49	0.53	4.97	0.01	0.02	0.96	0.00
Fornix / Stria terminalis L	2.81	0.06	0.05	0.79	0.04	12.72	0.00	0.00	0.99	0.00
Superior longitudinal fasciculus R	0.47	0.63	0.72	0.65	0.34	1.29	0.28	0.28	0.80	0.12
Superior longitudinal fasciculus L	0.61	0.55	0.67	0.30	0.47	1.32	0.27	0.12	0.51	0.27
Superior fronto-occipital fasciculus R	3.12	0.04	0.22	0.39	0.01	0.83	0.44	0.23	0.74	0.32
Superior fronto-occipital fasciculus L	4.61	0.01	0.48	0.08	0.00	1.50	0.22	0.26	0.69	0.09
Uncinate fasciculus R	1.82	0.16	0.06	0.12	0.67	1.36	0.26	0.11	0.48	0.28
Uncinate fasciculus L	1.05	0.35	0.24	0.16	0.82	5.95	0.00	0.01	0.93	0.00
Tapetum R	1.63	0.20	0.39	0.07	0.34	0.66	0.52	0.71	0.27	0.46
Tapetum L	2.17	0.12	0.27	0.04	0.35	0.02	0.98	0.88	0.87	1.00

Supplementary Table 2 Associations between ROI averaged FW / FA_t and ventricle volume in the three groups of participants

Tract	Controls		Siblings		Patients	
	r	*p*	*r*	*p*	*r*	*p*
ROI averaged FW						
Splenium of corpus callosum	0.36	0.0001	0.22	0.0045	0.42	0.0000
Fornix (column and body)	0.51	0.0000	0.60	0.0000	0.51	0.0000
Fornix / Stria terminalis R	0.33	0.0002	0.41	0.0000	0.39	0.0000
Fornix / Stria terminalis L	0.31	0.0005	0.44	0.0000	0.32	0.0000
Superior fronto-occipital fasciculus L	0.42	0.0000	0.39	0.0000	0.50	0.0000
ROI averaged FA_t						
Body of corpus callosum	-0.35	0.0001	-0.38	0.0000	-0.38	0.0000
Fornix (column and body)	-0.46	0.0000	-0.60	0.0000	-0.58	0.0000
Posterior limb of internal capsule L	0.28	0.0021	0.36	0.0000	0.30	0.0002
Retrolenticular part of internal capsule R	0.43	0.0000	0.28	0.0002	0.38	0.0000
Retrolenticular part of internal capsule L	0.27	0.0031	0.27	0.0004	0.25	0.0019

Supplementary Table 3 Secondary analysis including ventricle volume as an additional covariate in group-comparisons of the significant results of the ROI-based analyses

Tract	Group main effect on FW		post-hoc *p* values		
	F	*p*	Con vs. Sch	Con vs. Sib	Sib vs. Sch
uncinate fasciculus	5.14	0.006	0.008	0.79	0.005
superior corona radiata	4.32	0.014	0.005	0.25	0.041
fornix / stria terminalis	6.57	0.002	0.004	0.94	0.001

Summary and discussion

6

In this final chapter, I will summarize the main findings of projects presented in this thesis, provide a discussion of our main results in the context of existing literature, raise awareness about potential limitations, and bring up suggestions for future research.

Summary

Chapter 2 and 3 were based on a dataset of first-episode medication naïve schizophrenia patients, including a subgroup of patients with auditory verbal hallucinations (AVH), a group of patients without AVH, as well as healthy controls. Chapter 2 examined intrinsic functional connectivity among regions implicated in AVH by previous studies (Jardri et al., 2011; Kompus et al., 2011; Kühn and Gallinat, 2010), including the superior and middle temporal gyrus, inferior parietal lobule, inferior frontal gyrus, anterior cingulate cortex, insula and postcentral gyrus. Group-comparison of connectivity among these regions showed higher functional connectivity in a neural circuit comprising the right anterior cingulate cortex, right insula, and language processing areas (i.e. left superior temporal gyrus, left inferior parietal lobule) in patients with AVH as compared to both controls and non-AVH patients. Moreover, connectivity between the bilateral postcentral gyrus and left inferior frontal gyrus and right anterior cingulate cortex was higher in all patients as compared to controls, but with more pronounced alterations in the AVH patients than the non-AVH patients. These results suggest that AVH are related to a higher level of intrinsic functional interaction between the auditory cortex and associated brain regions, in the absence of confounding effects of prolonged illness and antipsychotic medication. The group-specific connectivity alterations in the AVH and non-AVH patients may underlie their dissociation in terms of behavioral symptoms.

Chapter 3 focused on interhemispheric connectivity abnormalities in relation to AVH in the same dataset, as schizophrenia patients have also been suggested to show altered functional and structural connectivity between homotopic brain regions in the two hemispheres, referred to as interhemispheric connectivity (Guo et al., 2013, 2014; Hulshoff Pol et al., 2004; Patel et al., 2011; Whitford et al., 2010). Our results showed that AVH patients have lower interhemispheric connectivity as compared to both controls and non-AVH patients in the anterior cingulate cortex and superior parietal lobule. Connectivity strength of these regions in patients correlated with their PANSS symptom scores. In addition, AVH patients showed lower interhemispheric connectivity in the precuneus and anterior cerebellum, and higher connectivity in the inferior frontal gyrus, while non-AVH patients were characterized by altered interhemispheric connectivity in the superior temporal gyrus and precentral gyrus as compared to controls. Both groups of patients showed lower interhemispheric connectivity in the parahippocampal gyrus and higher connectivity in the striatum than controls. These results indicate that schizophrenia patients are characterized

by interhemispheric connectivity abnormalities, and that the localization of affected regions may distinguish patients with and without AVH.

To further examine interhemispheric connectivity abnormalities in schizophrenia, chapter 4 examined the contribution of familial risk for schizophrenia to abnormalities in interhemispheric connectivity and hemispheric specialization in a large group of patients, their non-psychotic siblings and healthy controls. Interhemispheric connectivity was calculated as the level of connectivity between homotopic brain regions, and hemispheric specialization was defined as the difference in connectivity strength between intrahemispheric and heterotopic interhemispheric connections of each brain region. Hemispheric functional specialization quantifies the tendency of a region to favor within- over cross-hemispheric heterotopic connectivity. We found that schizophrenia patients have lower interhemispheric connectivity as well as higher hemispheric specialization as compared to controls and siblings. Moreover, changes in interhemispheric connectivity was correlated negatively with alterations in hemispheric specialization in patients relative to both controls and siblings. We did not find significant group-differences in connectivity measurements between siblings and controls, suggesting that abnormalities in interhemispheric connectivity and associated hemispheric specialization are more likely to be disease manifestation effects than familial risk effects.

Chapter 5 focused on structural connectivity derived from dMRI scans in schizophrenia patients, their non-psychotic siblings, and healthy controls. We applied the free-water imaging technique developed by Pasternak and colleagues (Pasternak et al., 2009), which computes the free-water fraction (FW), reflecting free-water accumulation in extracellular space, and the fractional anisotropy of the tissue compartment (FA_t), serving as a proxy for white matter integrity. Our results showed that patients have a higher level of FW in the left uncinate fasciculus, left superior corona radiata, and left fornix / stria terminalis as compared to controls and siblings. Siblings did not show a significant difference in FW as compared to controls, but did show an overall higher level of FA_t as compared to controls and patients. These results are consistent with previous findings of higher FW in schizophrenia patients (Lyall et al., 2018; Pasternak et al., 2012), and further suggest that the FW abnormalities identified in patients are likely to be expressions of established disease rather than related to familial risk for the illness. Our finding of higher levels of FA_t in siblings are suggestive of compensatory brain changes, possibly reflecting a protective mechanism to prevent individuals with familial risk for schizophrenia from developing psychosis.

Discussion

Functional dysconnectivity in patients with AVH

The results of chapter 2 and 3 show that schizophrenia patients with AVH have increased spontaneous functional connectivity among auditory cortex and associated brain regions, alongside decreased interhemispheric connectivity in these and other brain regions. As these two studies are based on first-episode, medication-naïve patients, our findings likely reflect an integral part of disease pathology, free from confounding factors such as medication and illness progression. One of the regions that was identified in both chapters is the anterior cingulate cortex, which shows higher functional connectivity with the superior temporal gyrus and insula (chapter 2), and reduced interhemispheric connectivity (chapter 3) in AVH patients specifically. The anterior cingulate cortex has been suggested to be involved in cognitive monitoring. Dysconnectivity of this region with the superior temporal gyrus may thus lead to abnormalities in differentiating sources of sounds, which may contribute to AVH symptoms in schizophrenia patients (Mechelli et al., 2007). Moreover, the anterior cingulate cortex has rich connections with the insula, and both regions have been implicated in first-episode patients and clinical high-risk individuals (Allen et al., 2012; Fusar-Poli et al., 2012; Moseley et al., 2013; Radua et al., 2012).

The results of chapter 2 also implicate other connectivity alterations that are most prominently altered in patients with AVH, and to a lesser extent in non-AVH patients. These abnormalities include connectivity between the inferior frontal cortex and bilateral postcentral gyrus. These regions have been reported in schizophrenia patients in previous studies (DeLisi, 2001), and our findings suggest that the severity of connectivity abnormalities in these regions may be related to the presence or absence of AVH symptoms. Note that we did not observe significant group-differences between the two groups of patients on PANSS negative and general pathology symptom scores, so it is unlikely that the dysconnectivity is simply related to illness severity.

Although we observed alterations in speech processing regions, we did not find abnormal connectivity between speech production areas in the left inferior frontal gyrus and speech perception regions in the left superior temporal cortex as implicated by some previous studies (Hubl et al., 2004; Lawrie et al., 2002). One possible reason for not replicating these previous results is that our sample size in chapter 2 and 3 was relatively small, and we may thus have lacked sufficient power to detect this group-effect. Moreover, as conclusive evidence for intrinsic functional connectivity alterations between the left inferior frontal gyrus and left superior temporal cortex in AVH patients is still lacking (Clos et al., 2014), more research is needed to establish whether, and if so how, putative connectivity abnormalities between speech-processing and speech-perception regions contribute to AVH symptoms in schizophrenia.

Interhemispheric connectivity in schizophrenia

Chapter 3 and 4 investigated interhemispheric functional connectivity abnormalities in relation to psychotic symptoms and familial risk for schizophrenia. The results suggest that schizophrenia patients as a group show altered functional connectivity between the two hemispheres, with different areas being implicated in AVH versus non-AVH patients. Moreover, the identified abnormalities seem to be effects of illness manifestation, rather than the expression of familial risk.

Homotopic interhemispheric connectivity, measuring functional synchronization between the two hemispheres, reflects an important aspect of brain network organization. Homotopic brain regions are directly connected through the corpus callosum, the largest white matter fiber bundle in the brain, to enable fast information transfer between the two hemispheres (Innocenti, 1995). Interhemispheric connectivity is the strongest type of functional interaction between brain regions (Stark et al., 2008; Zuo et al., 2010), higher than what would be predicted by anatomical distance alone (Salvador et al., 2005). Functionally, interhemispheric connectivity has been suggested to facilitate attentional resources in the face of demanding task requirements (Banich, 1998). Alterations of functional and structural interhemispheric connectivity in schizophrenia may disrupt the coordination between the two hemispheres and consequently affect the functional brain network to a larger extent (Guo et al., 2013, 2014; Hulshoff Pol et al., 2004; Patel et al., 2011; Whitford et al., 2010)

A long-standing hypothesis in schizophrenia suggests that schizophrenia symptoms may result from a failure to establish lateralization in language processing areas due to “transcallosal misconnection” (Crow, 1998, 1997). In support of this theory, neuroimaging studies have reported reduced lateralization in the temporal cortex (Oertel-Knöchel et al., 2014; Sommer et al., 2001). A lower level of interhemispheric connectivity in schizophrenia patients has also been reported: structural connectivity studies in general found lower FA in the corpus callosum in patients, but their results varied in terms of the specific section of corpus callosum being affected (Hulshoff Pol et al., 2004; Innocenti et al., 2003; Kubicki et al., 2008; Patel et al., 2011; Rotarska-Jagiela et al., 2008; Whitford et al., 2010). Functional connectivity studies also reported lower interhemispheric connectivity in a range of regions, including but not limited to areas identified in chapter 3 and 4 (Guo et al., 2014; Hoptman et al., 2012; Li, Xu, Zhang, Hoptman, & Zuo, 2015; Mwansisya et al., 2013). The wide range of regions implicated in schizophrenia may partly relate to the heterogeneous clinical profiles of recruited patients. The results of chapter 3, showing that interhemispheric connectivity of different brain regions may differentiate patients with and without AVH, support this theory.

To explore the relationship between interhemispheric connectivity and the pattern of intra- versus inter-hemispheric connectivity of a brain region, we adopted a newly proposed measurement called hemispheric specialization in chapter 4, following previous studies

(Mueller et al., 2015; Wang et al., 2014). We observed significant negative correlations between decreased interhemispheric connectivity and increased hemispheric specialization in patients as compared to controls and siblings, indicating that regional changes in interhemispheric connectivity are associated with alterations in the distribution of intra- and inter-hemispheric connectivity profiles of those brain regions in schizophrenia patients. Consistent with this finding, a previous study showed that interhemispheric connectivity accounts for 76% of group-differences in heterotopic and intra-hemispheric connectivity between patients and controls (Zhang et al., 2015). Taken together, these results suggest an important role for interhemispheric connectivity in functional brain network organization, and suggest that abnormalities in brain network organization in schizophrenia may, in part, be driven by disease-related changes in interhemispheric connectivity.

Structural dysconnectivity in schizophrenia

Chapter 5 examined structural connectivity abnormalities in schizophrenia using the free-water imaging method (Pasternak et al., 2009). The free-water imaging technique was designed to reduce the influence of extracellular free-water in tensor fitting, which is particularly relevant in white matter near the ventricles (Alexander et al., 2001; Pierpaoli and Jones, 2004). Free-water imaging has been suggested to increase the specificity of tissue modelling (Bergamino et al., 2017) and to improve test-retest reproducibility (Albi et al., 2017) as compared to single-tensor modelling techniques. Originally, the free-water fraction was merely considered a nuisance factor but it turned out to be meaningful imaging measure on its own. Free-water imaging studies of schizophrenia have shown that patients are characterized by lower fractional anisotropy (FA_t) in the tissue compartment and a higher free-water fraction (FW) putatively reflecting accumulation of free water in the extracellular space. The extent of identified abnormalities may depend on the stage of illness (Lyall et al., 2018; Oestreich et al., 2017; Pasternak et al., 2012, 2015). Specifically, first-episode patients tend to show higher FW and limited tissue changes (Lyall et al., 2018; Pasternak et al., 2012), whereas patients in the chronic phase exhibit more widespread reductions in FA_t, which are suggestive of reduced white matter integrity (Oestreich et al., 2017; Pasternak et al., 2015). Patients recruited in chapter 5 were mostly beyond the first-episode stage, but with a relatively short illness duration (median duration of illness of 4 years). Correspondingly, the extent of white matter abnormalities observed in our study appears to fall in between those reported in first-episode patients (Lyall et al., 2018; Pasternak et al., 2012) and chronic patients with a much longer illness duration (Oestreich et al., 2017; Pasternak et al., 2015).

It is of great interest to determine the underlying mechanism of higher FW in schizophrenia. Biological mechanisms including neuroinflammation and neurodegeneration

may contribute to higher levels of FW in patients (Pasternak et al., 2016; Syková and Nicholson, 2008). Neuroinflammation may lead to higher FW, and previous studies have implicated neuroinflammation in the pathology of schizophrenia (Pasternak et al., 2016). A putative association between neuroinflammation and ventricular enlargement is supported by studies showing a relationship between proinflammatory gene polymorphisms and ventricle size (Papiol et al., 2005), and by findings that fetal exposure to proinflammatory cytokines correlates with ventricular volume in adulthood (Ellman et al., 2010). Alternatively, neurodegeneration due to reduced dendritic spine count (Bennett, 2011; Glantz and Lewis, 2000; Kolluri et al., 2005) and cell density (Sweet et al., 2003; Uranova et al., 2004) could lead to concomitant extracellular space increases and ventricular enlargement in patients. Further investigations of the biological mechanism underlying FW accumulation and ventricular enlargement may help clarify the etiology of schizophrenia.

There is also a possibility that higher FW is related to enlarged ventricle in patients due technical issues such as misregistration. Misregistration may cause ventricle voxels to spill over into the white matter skeleton, which can lead also result in higher FW. To limit possible signal contamination due to spill-over, we used a conservative voxel selection method based on the intersection of white matter skeleton and the JHU ICBM-DTI-81 atlas. Thus, only voxels in the center of the skeleton entered into our analyses. In addition, we performed a secondary analysis in which we added ventricle volume as an additional regressor in the group-comparisons, which showed that group-differences remained statistically significant when controlling for ventricular volume. Together, the results of these analyses suggest that higher FW is unlikely to be solely due to ventricle enlargement in patients.

Contribution of familial risk for schizophrenia to functional and structural connectivity

Studying non-psychotic siblings of schizophrenia patients provides a valuable opportunity to determine the contribution of familial risk for schizophrenia to neural abnormalities observed in patients. The risk for schizophrenia in first-degree relatives of patients is around ten times higher than in individuals with no affected relatives (Lichtenstein et al., 2009; Mortensen et al., 1999). As promising disease endophenotype candidates, brain imaging measurements are examined in patients' non-psychotic siblings to elucidate the pathway from familial risk to clinical disease manifestation (Gottesman and Gould, 2003). However, so far, sibling studies have provided inconsistent results in terms of adopted neuroimaging measurements and the location of reported brain changes (Honea et al., 2008; MacDonald et al., 2009; Mothersill et al., 2012).

In chapter 4 and 5, we did not find a significant contribution of familial risk to the examined connectivity measurements, despite including a relatively large sample of non-

psychotic siblings. Our negative findings in the siblings may be due to several reasons. First, schizophrenia risk genes may confer premorbid disease alterations in specific connections, which may not stand out in whole-brain analyses. For example, a previous study compared grey matter volume in a large sample of 169 patients with a schizophrenia spectrum disorder, 213 of their unaffected siblings, and 212 healthy controls, and found siblings to have a trend level volume decreases in the medial frontal, superior temporal, insular cortex, but these effects did not survive whole-brain multiple testing correction (Honea et al., 2008). Second, as discussed in chapter 5, compensatory changes may occur in non-psychotic siblings during development, in response to early insults to the neural system (Mizuno et al., 2016). In support of this hypothesis, some preliminary evidence showed a delayed development in siblings of childhood-onset schizophrenia, who exhibit reduced corticocortical connectivity in childhood but normalized connectivity when reaching adulthood (Zalesky et al., 2015). In addition, in a study of clinical high-risk subjects, individuals who exhibited improvements of sub-threshold symptoms at follow-up were found to show increased fiber integrity in the corpus callosum (Katagiri et al., 2015). In all, while our general lack of findings in unaffected siblings suggests that the connectivity changes we observed in patients are related to disease manifestation rather than familial risk, we cannot rule out the possibility that connectivity abnormalities were present in siblings earlier in their development but that these abnormalities may be normalized by the time of their inclusion in our study.

Limitations

Imaging methods

There are several points that merit consideration when interpreting the results of this thesis. Methodologically, although fMRI is widely applied in exploring functional network organization in health and disease, the biological mechanisms underlying the spontaneous fMRI signal remain to be fully elucidated. Previous studies have suggested the fMRI signal to be correlated with glutamate synaptic activity and recycling (Raichle and Mintun, 2006), and neurophysiological evidence has pointed out that spontaneous fMRI signals may be related to neural firing rates and gamma local field potentials (Nir et al., 2008). Moreover, recent imaging genetic studies have shown that functionally connected brain regions express similar genes (Richiardi et al., 2015). These studies have laid a foundation for explorations of the biological basis of the fMRI signal, but currently, we cannot readily link macroscopic connectivity alterations observed in schizophrenia to putative microscopic cellular abnormalities.

Likewise, diffusion measurements derived from diffusion-weighted MRI (dMRI) are regarded as a proxy of microstructural characteristics of white matter. Diffusion

measurements are computed based on the water diffusion profile in brain tissue, which is influenced by many factors, such as axon diameters, alignment, myelination (Jones, 2010). Moreover, issues as partial volume effects and crossing fibers can also affect the size and shape of the diffusion profile. Free-water imaging is among recent attempts to improve tissue modelling with dMRI techniques (Pasternak et al., 2018). As shown in previous studies and in chapter 5, free-water imaging can distinguish between white matter fiber impairments and free-water accumulation, which may be prominent at different stages of illness in schizophrenia (Lyall et al., 2018; Oestreich et al., 2017; Pasternak et al., 2015, 2012).

Beyond the abovementioned difficulties, both fMRI and dMRI suffer from issues in image formation, such as magnetic field inhomogeneities, physiological noise contamination, and effects of head motion, which reduce the signal to noise ratio of images. Such disadvantages are hard to eliminate, but with careful experiment implementation and pre-processing correction, we were able to limit the influence of these factors to our results to a large extent.

Characteristics of recruited patients

Schizophrenia is a heterogeneous disorder, characterized by multiple additive genetic risk variants, widespread neural abnormalities, and different clinical profiles and outcomes (Brugger and Howes, 2017; Keshavan et al., 2011). Many studies have been performed to stratify patients into subgroups based on biological characteristics. For example, studies have shown that there are distinct psychosis biotypes across clinical diagnosis boundaries in patients with schizophrenia, schizoaffective disorder, and bipolar disorder with psychosis (Clementz et al., 2016). Other studies have suggested that schizophrenia patients can be categorized into two groups: deficit patients, characterized by more pronounced negative symptoms and persistent impairment, and nondeficit schizophrenia patients (Strauss et al., 2010). These two subgroups of patients showed group-difference in cortical thickness and brain network organization (Wheeler et al., 2015). Our results in chapter 2 and 3 of this thesis, showing that patients with and without AVH have distinct functional connectivity abnormalities, add to efforts to identify the neurobiological abnormalities underlying different behavioral symptoms of schizophrenia patients.

Another point that merits consideration when interpreting the findings in this thesis is the effect that antipsychotic medication may have on brain structural and functional connectivity (Bolding et al., 2012; Konopaske et al., 2008; Lesh et al., 2015). Previous studies have reported both increased and decreased functional connectivity changes in patients after short-term exposure to antipsychotics, including changes in connectivity between prefrontal, subcortical, and limbic systems (Bolding et al., 2012; Kraguljac et al., 2016; Sarpal et al., 2015), among default mode network regions (Sambataro et al., 2010), as

well as among other cortical and subcortical regions (Lui et al., 2010). These effects may depend on the type, dosage, and exposure length of medication intake and it is unclear whether these changes reflect disruptions of connectivity caused by psychotropic medication, or a normalization of disease-related connectivity changes (Abbott et al., 2013). In chapter 4 and 5, most patients were receiving antipsychotic medication at the time of recruitment. Although we did not find a correlation between medication dosage and brain connectivity measurements in patients, we cannot rule out the possibility that psychotropic medication use may have influenced our results.

Study design

Another limitation of the studies in this thesis is the cross-sectional study design and univariate statistical analyses that we adopted. The cross-sectional study design is unable to capture dynamic changes of neural abnormalities in schizophrenia patients and their siblings. Schizophrenia has been suggested to involve both neurodevelopmental and neurodegenerative changes (Cannon et al., 2003; Hulshoff Pol and Kahn, 2007). Follow-up studies of clinical or genetic high-risk individuals can help uncover premorbid alterations which have predictive value for conversion to psychosis and to develop preventive strategies (Collin et al., 2018; van Haren et al., 2008). Longitudinal studies following patients from the first-episode to the chronic stage may help to elucidate continuous neurobiological and clinical changes in patients.

Future perspectives

The findings in this thesis add some new insights into schizophrenia-related neural abnormalities, but also raise many questions for further exploration.

The results of chapter 5 indicate that patients' non-psychotic siblings may exhibit an overall higher level of FA_t than patients and controls, and we did not find evidence for significantly lower functional and structural connectivity in siblings as compared to their affected probands (chapter 4 and 5). These findings are somewhat unexpected given that the level of heritability of schizophrenia is estimated to be 67 ~ 81% (Cardno and Gottesman, 2000; Hilker et al., 2018), and focal brain abnormalities have been reported in siblings, especially in frontal cortex, temporal areas and hippocampus (Boos et al., 2007; Honea et al., 2008; Mothersill et al., 2012). Future studies incorporating genetic information (genotype, methylation, and gene expression data) together with MRI measurements in patients and their relatives will be informative to clarify the pathophysiological pathway from genetic risk to imaging phenotypes and behavioral symptoms (Elliott et al., 2018; Meyer-Lindenberg, 2010; Thompson et al., 2014). For example, schizophrenia candidate gene catechol-O-methyltransferase (COMT) was shown to have a significant effect on the FA,

axial and radial diffusivities of white matter fibers in healthy children and adolescents (Thomason et al., 2010). Similarly, genetic variation in the ZNF804A genotype has been shown to modulate functional connectivity between the dorsal prefrontal cortex and hippocampus in both schizophrenia patients and siblings (Rasetti, 2011). Moreover, polygenic effects of schizophrenia risk variants were also shown to correlate with total brain and white matter volume (Terwisscha van Scheltinga et al., 2013). Future imaging genetic studies may help establish a link from genetic risk to neural abnormalities and clinical manifestations of schizophrenia.

Another question worthy of further examination is the relationship between brain regions' lateralization of task-induced activation and their tendency to connect with other regions in the same hemisphere during rest (i.e. hemispheric specialization as examined in chapter 4). It has been suggested that schizophrenia patients show lower structural and functional lateralization as compared to controls. However, it is unknown how reduced lateralization relates to hemispheric specialization impairments if we believe that the function of a brain region is partially dependent on its connectivity pattern (Eickhoff et al., 2015). Our results of higher hemispheric specialization in patients during rest (chapter 4) and previous evidence of impaired lateralization of task induced activation suggest that these are associated abnormalities. However, a study acquiring both task and resting-state fMRI will be required to directly test the relationship between these two measurements.

Longitudinal studies of schizophrenia using the free-water imaging technique will provide information on dynamic changes of free-water concentration and tissue integrity in patients. As discussed in chapter 5 and previous studies (Pasternak et al., 2015, 2012), both neurodegeneration and neuroinflammation may contribute to free-water accumulation in patients, but the timing of these two processes may differ. If higher FW in patients is mainly due to neurodegeneration resulting from cell death, loss of synaptic contact etc., then we would expect to observe an exacerbation of FW accumulation with prolonged illness. However, if neuroinflammation is the main cause, we would expect higher FW near disease outbreaks, followed by an increased level of inflammatory response and reduced FW in more chronic illness stages. Follow-up studies would be insightful to reveal which of these possible processes is more likely related to FW changes in schizophrenia.

Studies in this thesis employed univariate statistical analysis, which is suitable for region-based analysis. However, it does not consider interactions among tested measurements. Future studies using multivariate analyses can provide new insight into disease-related neural abnormalities. For example, independent component analyses have been used to identify brain network aberrances in schizophrenia and other neuropsychiatric disorders (Calhoun et al., 2001; Menon, 2011). In addition, multivariate classification analyses can distinguish schizophrenia patients from healthy subjects based on

neuroimaging features (Fan et al., 2008) and categorize schizophrenia patients into homogenous subgroups (Schnack, 2017). Advancements of such data analysis techniques may help to uncover potential illness biomarkers in schizophrenia.

Concluding remarks

The studies in this thesis aimed to examine structural and functional connectivity abnormalities in schizophrenia in relation to familial risk and to AVH as one of its cardinal symptoms. Our results show that patients have abnormalities in intrinsic functional connectivity patterns such as hemispheric integration and hemispheric specialization. Moreover, these abnormalities appear to differ in patients with and without AVH symptom, emphasizing that different neural mechanisms may exist in patients with distinct clinical profiles. In terms of structural connectivity, we found that schizophrenia patients show extracellular free-water accumulation in several white matter regions. We did not observe significant connectivity abnormalities in siblings in any of the connectivity measurements examined in this thesis. Taken together, using various neuroimaging techniques, we show abnormalities in both functional and structural cortico-cortical interactions in schizophrenia patients, and our findings suggest that these abnormalities are likely to be part of the biological substrate for behavioral symptoms of the illness, and expressions of disease manifestation rather than familial risk for schizophrenia.

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Nederlandse samenvatting

Het onderzoek in dit proefschrift richt zich op functionele en structurele connectiviteit in de hersenen berekend aan de hand van MRI-scans die zijn verzameld van schizofrenie patiënten, hun niet-aangedane broers en zussen en gezonde controles. Hiermee worden afwijkingen in connectiviteit in schizofrenie onderzocht in relatie tot symptomatologie en familiair risico op de aandoening.

Hoofdstuk 2 en 3 zijn gebaseerd op een dataset van eerste-episode medicatie-naïeve schizofrenie patiënten, bestaande uit een groep patiënten met auditief verbale hallucinaties (AVH), een groep patiënten zonder AVH en een groep gezonde proefpersonen. In Hoofdstuk 2 onderzochten we de intrinsieke functionele connectiviteit tussen hersengebieden waarvan eerdere studies hebben aangetoond dat ze betrokken zijn bij AVH (Jardri et al., 2011; Kompus et al., 2011; Kühn and Gallinat, 2010), zoals de superieure en mediale temporale gyrus, inferieure pariëtale cortex, inferieure frontale gyrus, anterieure cingulate cortex, insula en postcentrale gyrus. Onze studie liet zien dat in schizofrenie patiënten met AVH een hogere functionele connectiviteit tussen deze hersengebieden vertonen dan zowel controles als patiënten zonder AVH. Afwijkingen in connectiviteit werden voornamelijk gevonden in een netwerk bestaande uit de rechter anterieure cingulate cortex, rechter insula en gebieden betrokken bij taalverwerking, namelijk de linker superieure temporale gyrus en de linker inferieure pariëtale cortex. Bovendien was de connectiviteit tussen de postcentrale gyrus (beiderzijds) en de linker inferieure frontale gyrus en rechter anterieure cingulate cortex hoger bij alle schizofrenie patiënten in vergelijking met de gezonde proefpersonen, maar waren deze verschillen meer uitgesproken bij de AVH-patiënten dan bij de niet-AVH-patiënten. Deze resultaten suggereren dat AVH in schizofrenie gerelateerd zijn aan een hoger niveau van intrinsieke functionele interactie tussen de auditieve cortex en hersengebieden die betrokken zijn bij taalverwerking. Aangezien we dit aantoonden in een groep medicatie-naive patiënten in hun eerste episode kunnen we bovendien stellen dat deze afwijkende connectiviteit niet het gevolg lijkt van de effecten langdurige ziekte of antipsychotische medicatie. De groeps-specifieke veranderingen in connectiviteit in de AVH- en niet-AVH-patiënten kunnen een mogelijke verklaring zijn voor de verschillen in symptomatologie tussen de patienten.

Hoofdstuk 3 richt zich op mogelijke afwijkingen in interhemisferische connectiviteit in relatie tot AVH, gemeten in dezelfde dataset. Er zijn namelijk aanwijzingen dat de functionele en structurele connectiviteit tussen (homotopische) gebieden in de linker en rechter hersenhelft (aangeduid als interhemisferische connectiviteit) is aangedaan in schizofrenie (Guo et al., 2013, 2014; Hulshoff Pol et al., 2004; Patel et al., 2011; Whitford et al., 2010). Onze resultaten toonden aan dat AVH-patiënten een lagere interhemisferische

connectiviteit hebben in vergelijking met zowel controles als met niet-AVH-patiënten in het voorste deel van de cingulate cortex en de superieure pariëtale cortex. In de patiënten vertoonde de verbindingssterkte van deze hersengebeden een significante correlatie met hun symptomen, gemeten aan de hand van de Positive and Negative Syndrome Scale (PANSS). Bovendien vertoonden AVH-patiënten een lagere interhemispherische connectiviteit in de precuneus en het anterieure deel van het cerebellum, en een hogere connectiviteit in de inferieure frontale gyrus. Niet-AVH-patiënten werden daarentegen gekenmerkt door een veranderde interhemisferische connectiviteit in de superieure temporale gyrus en precentrale gyrus. Beide patiënten groepen vertoonden lagere interhemisferische connectiviteit in de parahippocampale gyrus en hogere connectiviteit in het striatum in vergelijking met de gezonde proefpersonen. Deze resultaten suggereren dat schizofreniepatiënten worden gekenmerkt door afwijkingen in interhemisferische connectiviteit en dat de locatie van aangedane hersengebieden mogelijk betrokken is bij het onderscheid tussen patiënten met en zonder AVH.

In hoofdstuk 4 keken we naar de mogelijke bijdrage van een familiair risico op schizofrenie op afwijkingen in interhemisferische connectiviteit en hemisferische specialisatie. Hiervoor maakten we gebruik van functionele MRI scans van een grote groep schizofrenie patiënten, hun niet-aangedane broers en zussen en gezonde proefpersonen. Interhemispherische connectiviteit werd berekend als de mate van connectiviteit tussen homotopische hersengebieden. Hemisferische functionele specialisatie is een maat van de verhouding tussen intrahemisferische en interhemisferische heterotopische connectiviteit, en werd berekend als het verschil in verbindingssterkte tussen intrahemisferische en en interhemisferische heterotopische verbindingen van elk hersengebied. We vonden dat patiënten met schizofrenie een lagere interhemisferische connectiviteit en een hogere mate van hemisferische specialisatie lieten zien in vergelijking met zowel gezonde proefpersonen als niet-aangedane broers en zussen. Bovendien waren veranderingen in interhemisferische connectiviteit negatief gecorreleerd met veranderingen in hemisferische specialisatie bij schizofrenie patiënten in vergelijking met gezonde proefpersonen en broers en zussen. We vonden geen significante groepsverschillen in deze connectiviteitsmaten tussen de gezonde proefpersonen en de niet-aangedane broers en zussen van schizofrenie patiënten. Dit suggereert dat afwijkingen in interhemisferische connectiviteit en hemisferische specialisatie waarschijnlijk direct gerelateerd zijn aan het hebben van de ziekte en niet zozeer gekoppeld zijn met familiaire risicofactoren.

Hoofdstuk 5 richtte zich op structurele connectiviteit berekend op basis van diffusie-gewogen MRI scans die werden verzameld bij schizofreniepatiënten, hun niet-aangedane broers en zussen en gezonde proefpersonen. Hier werd de zogenaamde ‘Free-water’ methode toegepast (Pasternak et al., 2009), die de vrij-water fractie (FW) berekent die

geassocieerd is met vrij water in de extracellulaire ruimte, en de voor FW gecorrigeerde fractionele anisotropie (FAt). Hogere FW is mogelijk een uiting van neuroinflammatie terwijl FAt vaak wordt gebruikt als een benadering van de algemene kwaliteit (integriteit) van witte stof verbindingen in de hersenen. We vonden hogere FW waarden voor patiënten (in vergelijking met gezonde proefpersonen en niet-aangedane broers en zussen) in de linker arcuate fasciculus, de linker superieure corona radiata en de linker fornix/stria terminalis. Voor de broers en zussen van patiënten werden geen verschillen in FW gevonden ten opzichte van gezonde proefpersonen maar ze lieten wel een hogere globale FAt waarde zien ten opzichte van patiënten en gezonde proefpersonen. De FW resultaten komen grotendeels overeen met eerdere bevindingen (Lyall et al., 2018; Pasternak et al., 2012) en suggereren dat hogere FW waarden in patiënten een gevolg zijn van het hebben van de ziekte en niet gekoppeld zijn aan familiair risico op schizofrenie. De hogere FAt gevonden in de broers en zussen wijst op een mogelijk compensatie mechanisme dat voorkomt dat mensen met een familiair risico op schizofrenie een psychose ontwikkelen.

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List of publications

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Chang, X., Collin, G., Xi, Y., Cui, L., Scholtens, L.H., Sommer, I.E., Wang, H., Yin, H., Kahn, R.S., et al., 2017. Resting-state functional connectivity in medication-naïve schizophrenia patients with and without auditory verbal hallucinations: A preliminary report. Schizophr. Res. 188, 75–81.

Chang, X., Collin, G., Mandl, R.C.W., Cahn, W., Kahn, R.S., 2019. Interhemispheric connectivity and hemispheric specialization in schizophrenia patients and their unaffected siblings. NeuroImage Clin. 101656.

Chang, X., Mandl, R.C.W., Pasternak, O., Brouwer, R.M., Cahn, W., Collin, G., Kahn, R.S. Diffusion MRI Derived Free-Water Imaging Measures in Schizophrenia Patients and Their Non-Psychotic Siblings. In submission.

Curriculum Vitae

Xiao Chang was born on July 6th, 1989 in Xi'an, China. She studied Material Physics at Northwestern Polytechnical University for her Bachelor from 2007 to 2011. After that, she started her Master's program in Psychology at Forth Military Medical University. In 2014, she came to the Netherlands for her PhD project under the supervision of Prof Kahn at the Department of Psychiatry, University Medical Center Utrecht. Her PhD project uses magnetic resonance imaging techniques to examine functional and structural connectivity in schizophrenia in relation to auditory verbal hallucinations and familial risk for the disease. She is now continuing her research at King's College London on identification of neurobiological substrates related to psychopathology in adolescents.