An Exploration of Functional Connectivity and GABA in Schizophrenia and Related Conditions

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Summary

Schizophrenia is a severe and enduring mental illness with psychopathology including positive symptoms, negative symptoms and cognitive impairment. It has been hypothesised that such symptoms represent a loss of integration within and between brain regions. This is known as the dysconnectivity hypothesis and integrates with other neurochemical hypotheses of the disorder.

In this thesis, I sought to explore the dysconnectivity hypothesis using amplitude envelope correlation in MEG, firstly in two groups of individuals with schizophrenia. I then sought to address the continuum model of schizophrenia through exploring functional connectivity in two groups with high schizotypy. Next, I explored dysconnectivity in schizophrenia in more depth by looking separately at individuals with recent onset psychosis and those with established schizophrenia. I then went on to look at connectivity following ketamine administration thus seeking to link this model of schizophrenia with my findings in those with schizophrenia. Finally, I explored the GABA hypothesis of schizophrenia using MRS, again in two groups of individuals with schizophrenia, at different stages of illness and linked this with connectivity.

Overall, this work supports the dysconnectivity hypothesis of schizophrenia, finding reduced connectivity in schizophrenia. Such changes are found predominantly in the later stages of the disorder suggesting the possibility of progressive changes in connectivity throughout its course. I found increased connectivity following ketamine administration in the same frequency band and region suggesting the drug does not model later stages of the disorder well (where I predominantly found hypo-connectivity). In addition, I found reduced GABA

in later stages of schizophrenia but not in early stages, again suggesting progressive changes throughout the course of the disorder.

Finally, I also found hypo-connectivity in healthy volunteers with high schizotypy scores suggesting biological continuity between subclinical symptoms and diagnosable schizophrenia.

Overall, these results add support to the dysconnectivity hypothesis, the GABA/glutamate hypothesis and the continuum hypothesis of schizophrenia.

Statements

DECLARATION

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Signed (candidate) Date 25th January 2018

STATEMENT 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated, and the thesis has not been edited by a third party beyond what is permitted by Cardiff University's Policy on the Use of Third Party Editors by Research Degree Students. Other sources are acknowledged by explicit references. The views expressed are my own.

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I hereby give consent for my thesis, if accepted, to be available online in the University's Open Access repository and for inter-library loan, and for the title and summary to be made available to outside organisations.

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Data Collection

All analyses presented in this thesis were performed by me, using in-house scripts based around the Fieldtrip package.

The data presented in Chapter 3 was collected by Dr Laura Whitlow (Cardiff University) and the University of Nottingham MISP study team.

The data presented in Chapter 4 was collected as part of the UK MEG Partnership (MR/K005464/1) and 100 brains by staff at Cardiff University and the University of Nottingham.

The data presented in Chapter 5 was collected by me, together with Loes Koelewijn as part of MRC SPRING (The Study of Psychosis and the Role of Inflammation, GABA and Glutamate).

The pharmacological MEG data (13/WA/0059) presented in Chapter 6 was collected by Dr Suresh Muthukumaraswamy.

Aside from the data presented in Chapter 6, none of the other data presented in this thesis has been previously analysed. The resting-state ketamine data presented in Chapter 6 was previously analysed by Dr Suresh Muthukumaraswamy using a different analysis method.

Contents

Abbreviations

Abbreviation	Description
AAL atlas	Automated Anatomical Labelling atlas
ALFF	Amplitude of low frequency fluctuations
BOLD	Blood oxygen level dependent
CNV	Copy Number Variant
Cr	Creatine
CSF	Cerebrospinal fluid
CSI	Coherence Source Imaging
DCM	Dynamic Causal Modelling
DDD	Mean daily dose equivalents
DMN	Default Mode Network
dmPFC	Dorsomedial prefrontal cortex
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders (4th. Ed.)
DTI	Diffusor tensor imaging
FA	Fractional anisotropy
FE	First episode
fMRI	Functional magnetic resonance imaging
GABA	γ-Aminobutyric acid
GAD67	Glutamic acid decarboxylase
GM	Grey Matter
GMM	Gaussian mixture modelling
HRF	Haemodynamic response function
ICA	Independent Component Analysis

ICD-10	International Statistical Classification of Diseases and Related Health Problems
LPFC	Lateral prefrontal cortex
MEG	Magnetoencephalography
MINI	MINI-International Neuropsychiatric Interview
MPFC	Medial prefrontal cortex
MRBD	Movement related beta desynchronization ()
MRI	Magnetic Resonance Imaging
NMDA	*N*-methyl-D-aspartate
NMDAR	*N*-methyl-D-aspartate receptor
OCC	Occipital Lobe
PANSS	Positive and Negative Syndrome Scale
PCC	Posterior cingulate cortex
SANS	Scale for the Assessment of Negative Symptoms
SAPS	Scale for the Assessment of Positive Symptoms
SNR	Signal-to-noise ratio
SNV	Single nucleotide variants
SPQ	Schizotypal Personality Questionnaire
SQUID	Superconducting quantum interference device
T	Tesla
tDCS	Transcranial Direct Current Stimulation
TE	Echo time
TR	Repetition time
UHR	Ultra high risk (UHR) for psychosis
WM	White matter

Chapter 1 General Introduction

1.1 Schizophrenia

Schizophrenia is typically a severe and enduring mental illness with a prevalence of 0.4-0.7% (Saha et al. (2005). Its psychopathology includes positive symptoms such as delusions and hallucinations and negative symptoms such as apathy and avolition. More recently it has been recognised that cognitive impairment can be a prominent feature of schizophrenia with impairments identified in a wide range of cognitive processes, including executive function, working memory, attention and learning (Heinrichs and Zakzanis, 1998). Such cognitive impairment is poorly responsive to anti-psychotic medication and as a result can cause significant morbidity (Owen et al., 2016). It has been found to correlate with employment (Gold et al., 2002), functional outcome (Green et al., 2000) and independent living.

Diagnosis is made using diagnostic criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013) and the International Statistical Classification of Diseases and Related Health Problems (WHO, 1993). ICD-10 divides the disorder into paranoid, hebephrenic, undifferentiated, catatonic and residual schizophrenia, reflecting the heterogeneity of the disorder. Therefore, whilst criteria are standardised, patients can vary significantly in their presentation.

The disorder typically presents in adolescence and early adulthood with prodromal, subclinical symptoms prior to the onset of frank psychosis. However, studies of individuals at high risk of developing schizophrenia and longitudinal studies of children that later go on to develop the disorder suggest that there are impairments in multiple domains including neuromotor, receptive language and cognitive development even before the onset of the disorder (Cannon et al., 2002, Keshavan et al., 2005).

It has long been recognized that schizophrenia is a heterogeneous condition with its course varying according to nature of onset, pattern of psychotic episodes, psychopathology between episodes and also treatment response (Carpenter and Kirkpatrick, 1988, Tandon et al., 2013). For example, a review by Menezes et al. (2006) described 42% of patients having a "good outcome", 35% an "intermediate outcome" and 27% a "poor outcome". Prognosis is linked to response to treatment, the mainstay of which is with antipsychotic medication. Antipsychotics act by blocking dopamine receptors (Jones and Pilowsky, 2002), however, such treatments do not necessarily address the underlying aetiology of the disorder. Whilst most antipsychotics are effective in treating positive symptoms, they are less effective in treating negative and cognitive symptoms. In addition, up to 30% of patients with schizophrenia have residual symptoms after failing to respond to two different antipsychotics and are therefore classified as treatment resistant (Meltzer, 1997). It has been suggested that this differential response to treatment may be due to heterogeneity in aetiology.

Whilst the disorder is heterogeneous on multiple levels, the diagnosis often comes with a significant burden to sufferers, with medical comorbidity being much higher than the general population (Smith et al., 2013). Such medical co-morbidities are under-recognised which may contribute to the average life expectancy for sufferers being 10-20 years less than the general population (Smith et al., 2013, Chesney et al., 2014).

Recent advances in genomic technologies have led to significant improvements in our understanding of the genetic architecture of schizophrenia. The heritability of schizophrenia is around 80%, suggesting that a large proportion of susceptibility is inherited. It is now known that both common polymorphisms and rare variants such as Single Nucleotide Variants (SNVs) and Copy Number Variants (CNVs) contribute to schizophrenia susceptibility with varying effect sizes across a large number of genes (Rees et al., 2015).

Studies have also shown that a proportion of genetic risk of schizophrenia is not inherited but occurs de novo (Kirov et al., 2012).

At present, there are several challenges in the field of schizophrenia, the most significant being our limited understanding of aetiology and pathophysiological mechanisms underlying schizophrenia as well as the lack of effective, tolerable treatments for the disorder.

1.2 Aetiological Theories of Schizophrenia

There are multiple aetiological theories that seek to explain the development of schizophrenia, including the following:

1.2.1 Neurotransmitter hypotheses

1.2.1.1 The Dopamine Hypothesis

Initial aetiological theories of schizophrenia focussed upon the dopamine hypothesis which suggests that schizophrenia results from hyper-dopaminergia. Dopamine is a member of the catecholamine family with multiple projections through the brain, namely, nigrostriatal, mesolimbic and mesocortical pathways. Initial hypotheses suggesting hyper-dopaminergia as a mechanism in schizophrenia were developed after amphetamines (which increase dopamine release) were found to cause positive symptoms of schizophrenia. (Reviewed by Lieberman and Koreen (1993). In addition, it was discovered that dopamine receptor antagonists had antipsychotic actions that were related to their affinity for dopamine receptors (Seeman et al., 1976). The dopamine hypothesis was later modified to suggest that there is hyper-dopaminergia in subcortical regions and hypo-dopaminergia in pre-frontal regions (Davis et al., 1991). This resulted in the development of an antipsychotic, aripiprazole, which is a dopamine receptor partial agonist (Bowles and Levin, 2003). All other

licenced antipsychotics are dopamine receptor antagonists in addition to their actions at numerous other receptors (Mailman and Murthy, 2010). Whilst they are effective in treating positive symptoms of schizophrenia, they treat negative and cognitive symptoms poorly and therefore, it has been suggested that disruptions to dopamine alone do not fully explain the pathogenesis of schizophrenia. In addition, around a third of patients fail to respond adequately to such drugs and therefore other aetiological mechanisms have been sought (Elkis, 2007).

1.2.1.2 The GABA/Glutamate Hypothesis

GABA is the primary inhibitory neurotransmitter in the central nervous system. It is synthesised by Glutamic Acid Decarboxylase (GAD67), an enzyme that converts glutamate to GABA. Glutamate is the primary excitatory neurotransmitter in the central nervous system (Rothman et al., 2003). It acts upon three ionotropic receptors; NMDA, kainate and AMPA receptors and eight metabotropic receptors. In particular, the ionotropic NMDA receptor (NMDAR) has been implicated in schizophrenia. The NMDAR is important in synaptic plasticity, cortical development, learning and working memory (Collingridge et al., 2013), all of which are relevant in the psychopathology of schizophrenia.

In normal neuronal functioning, primary glutamatergic firing onto GABAergic interneurons results in reduced secondary glutamatergic tone and therefore reduced dopaminergic neuronal firing in mesolimbic pathways. It is purported that in schizophrenia, there is hypofunction at NMDA receptors attached to GABAergic interneurons and that this results in disinhibition of secondary glutamatergic neurons (Schwartz et al., 2012, Homayoun and Moghaddam, 2007).

Glutamate was initially implicated in the pathogenesis of schizophrenia due to the psychotogenic effects of dissociative anaesthetics such as ketamine which act by blocking the NMDAR. NMDAR antagonists block NMDA receptors on GABAergic interneurons resulting in excess glutamate release (Moghaddam and Krystal, 2012). This is supported by animal (Bustos et al., 1992, Moghaddam et al., 1997) and human (Rowland et al., 2005, Stone et al., 2012) studies finding increased glutamate following administration of NMDA receptor antagonists. It is postulated that hyperglutamatergia causes increased activation of dopaminergic neurones which leads to psychosis (Howes et al., 2015). NMDA receptor blockade has also been shown to alter D2 receptor binding and expression (du Bois et al., 2008). In addition, excess glutamate may also result in excitotoxicity and neuronal death. This has been postulated as a model of schizophrenia since such drugs exacerbate symptoms in patients with schizophrenia (Lahti et al., 1995) and mimic positive, negative and cognitive symptoms of schizophrenia in healthy controls (Adler et al., 1999, Krystal et al., 2005). In addition, over recent years it has been noted that sufferers of autoimmune NMDAR encephalitis, (involving antibodies targeted at the NMDAR) often experience symptoms of psychosis (Deakin et al., 2014).

GABA was first implicated in schizophrenia after multiple post-mortem studies of patients with the disorder showed reduced GAD67 mRNA and protein levels (Curley et al., 2011, Thompson et al., 2009). As discussed, GAD67 is the enzyme responsible for most cortical GABA production and GAD67 complete knockout mice show a 93% reduction in GABA and die within hours of birth (Asada et al., 1997). Other studies of mice with incomplete knockout of GAD67 show learning and social behaviour deficits (Zhang et al., 2014a) thus linking deficits in GABA synthesis with symptoms seen in schizophrenia. In other animal studies, density of GABAergic interneurons are reduced following the administration of NMDA antagonists (Braun et al., 2007, Keilhoff et al., 2004). This therefore links the

NMDAR hypofunction hypothesis and the GABAergic findings seen in schizophrenia. However, although post mortem studies clearly point towards disruption in GABA, in vivo studies of GABA using MRS in patients with schizophrenia are unclear with some studies showing a reduction in GABA, some showing an increase and some showing no change compared to healthy controls.

As discussed above, it is believed that the dopamine dysregulation seen in schizophrenia may be downstream of NMDA receptor dysregulation. SPECT and PET studies show enhanced striatal dopamine release in patients with schizophrenia in response to amphetamine (Laruelle et al., 1999). Similar results are found in healthy participants given ketamine (Kegeles et al., 2000) and in animal studies (Balla et al., 2001, Balla et al., 2002). This, again, therefore supports the NMDAR hypofunction hypothesis and suggests that dopamine dysregulation can also be explained by the model.

The involvement of GABA and glutamate pathways in the aetiology of schizophrenia is also supported by proteomic and genomic studies. Pathogenic CNVs in schizophrenia have been found to converge upon genes involved in glutamatergic and GABAergic neurotransmission (Pocklington et al., 2014) supporting hypotheses of the aetiology of schizophrenia implicating these neurotransmitter systems.

In addition, in vivo studies using Magnetic Resonance Spectroscopy (MRS) have found abnormal glutamate levels in schizophrenia (Marsman et al., 2013). Since glutamate is known to have excitotoxic effects when present in excess (Lau and Tymianski, 2010) it has been postulated that in the early stages of schizophrenia hyper-glutamatergia leads to excitotoxicity (Plitman et al., 2014). This is supported by a review of MRS studies by Poels et al. (2014) who found an increase in glutamatergic levels in the medial prefrontal cortex in early stage drug naïve patients with schizophrenia compared to controls. A meta-analysis by Marsman

et al. (2013) found that glutamate reduces with age in patients with schizophrenia suggesting possible neurochemical differences between early and later stages of the disorder. However, a meta-analysis by Merritt et al. (2016) found no association between glutamate levels and age or symptom severity in schizophrenia.

In summary, the NMDAR hypofunction hypothesis integrates the dopaminergic hypothesis of schizophrenia and also explains not only the positive symptoms of the condition but also the negative and cognitive symptoms. However, given that a significant proportion of patients with schizophrenia do not respond to antipsychotic medication (that primarily alter dopamine), it may be that patients with glutamate abnormalities represent distinct subtype of patients with schizophrenia to those with dopamine abnormalities (Egerton et al., 2012, Howes et al., 2015). Currently available antipsychotics (that primarily act upon dopamine) treat positive symptoms fairly well but do not treat negative and cognitive symptoms well. Such symptoms may therefore be better explained by glutamatergic perturbations

1.2.2 Dysconnectivity hypothesis

Connectivity refers to communication within and between brain regions. This can be divided into structural, functional and effective connectivity. Structural connectivity refers to the exploration of physical properties of white matter tracts within the brain and is often studied using an MRI technique known as diffusor tensor imaging (DTI). Functional connectivity refers to the correlation in neural activity over time in different brain regions and can be measured in vivo using multiple neuroimaging techniques such as fMRI, EEG and MEG. Effective connectivity refers to the directed influence of one brain region on the physiological activity in another brain region and can be explored using modelling techniques such as dynamic causal modelling (Friston, 1994).

It has been hypothesized that schizophrenia is a syndrome of dysconnectivity and that symptoms of the disorder result from abnormal communication within and between cortical networks (Friston and Frith, 1995, Pettersson-Yeo et al., 2011, Stephan et al., 2006, Stephan et al., 2009b, Friston et al., 2016). Synchronised oscillatory neural activity (as measured non-invasively in humans using Magnetoencephalography - MEG) is considered to be required for such communication. This oscillatory activity, particularly in the gamma frequency band, is dependent upon the interaction between excitatory (glutamatergic) and inhibitory (GABAergic interneuron) activity (Mann and Mody, 2009).

GABAergic interneurons can be subdivided into multiple types according to their gene expression (parvalbumin, somatostatin), morphology (basket cell, chandelier cell), electrophysiology and synaptic connectivity (Tremblay et al., 2016, Markram et al., 2004). The most common type is the parvalbumin expressing interneuron. Activation of fast-spiking parvalbumin (PV) interneurons has been found to be important in the generation of synchronous gamma oscillations (Cardin et al., 2009). PV interneurons (Hashimoto et al., 2008) and gamma oscillations have been found to be disrupted in schizophrenia (Uhlhaas and Singer, 2010). In addition, basket cells expressing cholecystokinin (CCK) have been found to be important in theta oscillations and are disrupted in schizophrenia (Curley and Lewis, 2012).

Given that neural oscillations require the synchronised activity of large groups of neurones, this provides a link between the dysconnectivity hypothesis of schizophrenia and neurochemical hypotheses implicating GABA and glutamate. Much of the work in schizophrenia has explored the gamma frequency band. High frequency gamma oscillations (30-90 Hz) occur in many cortical areas and are important for cognitive processing (Whittington et al., 2011). There is evidence of impaired high frequency synchronised oscillatory activity in patients with schizophrenia during perceptual, working memory and

executive tasks (Phillips and Uhlhaas, 2015). Therefore, in addition to providing a link between dysconnectivity and neurochemistry, abnormal oscillatory activity has the potential to provide a link between these and the impaired sensory processing and cognitive function of schizophrenia.

In addition to its role in gamma oscillations, inhibitory/excitatory balance has also been found to be important for oscillations in other frequency bands. Studies using GABA agonists in humans find reduced alpha power at rest (Schreckenberger et al., 2004, Ahveninen et al., 2007) and during working memory tasks (Lozano-Soldevilla et al., 2014), highlighting the importance of inhibitory tone for oscillations in this frequency band. It is believed that alpha oscillations are important in directed attention and that increased alpha power results in functional inhibition whereas decreased alpha power results in engagement (Frey et al., 2015). Through the direction of attention towards task relevant information and suppression of task irrelevant information, alpha modulation is believed to be important in cognitive processes such as working memory and therefore may clearly have an important role in schizophrenia (Kustermann et al., 2016, Abeles and Gomez-Ramirez, 2014). In addition to reduced alpha power and coherence (Stevens and Livermore, 1982), studies have found less efficient modulation of alpha in schizophrenia (Kustermann et al., 2016).

GABAergic activity has also been found to impact upon beta oscillations. Task based studies using GABA agonists have shown increased amplitude of baseline beta power (Hall et al., 2010) and increased movement related beta desynchronization (Hall et al., 2011) in healthy controls. Work by Muthukumaraswamy et al. (2013) exploring the GABA transporter agonist, Tiagabine, which increases endogenous GABA activity, also found increased baseline beta power and event related desynchronization but reduced post-movement beta rebound (PMBR) following administration of Tiagabine in healthy controls. Studies of schizophrenia have found abnormal beta oscillations during perceptual tasks (Uhlhaas et al.,

2006, Sun et al., 2013) and at rest (Venables et al., 2009). Therefore, oscillatory activity in multiple frequency bands has been found to be affected by excitatory/inhibitory balance and abnormalities implicated in schizophrenia.

It is considered that measurements such as power and frequency of gamma oscillatory activity as measured by MEG and EEG may be reflective of local connectivity, whilst low-frequency oscillations reflect long-distance integration (Donner and Siegel, 2011). However, in order to explore the dysconnectivity hypothesis of schizophrenia further, studies have investigated more widespread network connectivity across the brain using the methods outlined previously.

1.2.2.1 Structural Connectivity in Schizophrenia

Fractional anisotropy (FA) is a measure of white matter fibre integrity that has been found to be reduced in schizophrenia (Tamnes and Agartz, 2016, Cookey et al., 2014). A review of DTI studies by Tamnes and Agartz (2016) in early onset schizophrenia found mostly reduced FA in patients but no consistent region or degree. Deficits in white matter integrity in early stages of schizophrenia were also found in a review by Cookey et al. (2014). Attempts have been made to explore the impact of stage of illness, age and duration of onset in order to elucidate the progressive nature of such changes. Cross sectional studies looking at structural connectivity in both First Episode (FE) and chronic patients have found differences between the two. For example, Friedman et al. (2008) and Kong et al. (2011) both found significantly reduced FA in chronic patients but not in FE. White et al. (2011) found overall lower FA in patients which was more pronounced in chronic patients compared with FE. Other studies have found negative correlations between FA and illness duration (Carpenter et al., 2008).

A meta-analysis of diffusor tensor imaging (DTI) in schizophrenia by Yang et al. (2017) found an inverse relationship between FA and age in patients with schizophrenia which is reflected in studies of FA in healthy ageing (Grieve et al., 2007). This replicates work by Rosenberger et al. (2008), who found an age-related decline in FA in patients with schizophrenia in certain fibre tracts which was not evident in healthy controls. In addition to studies comparing FE and chronic schizophrenia, studies have also compared UHR groups with FE. In a longitudinal study by Carletti et al. (2012), UHR patients that later went on to develop psychosis showed progressive reductions in FA over time. Taken together, such studies suggest that there may be a progressive decline in structural connectivity throughout the course of schizophrenia. This fits with hypotheses and work in schizophrenia that suggests neurochemistry changes throughout the course of the disorder. However, most of these studies rely upon a cross sectional design and therefore are prone to confounding.

1.2.2.2 Functional connectivity

Over the last 20 years, the dysconnectivity hypothesis has been supported by multiple studies exploring functional connectivity in schizophrenia (Pettersson-Yeo et al., 2011). However, as with structural connectivity studies, results of from functional connectivity studies are heterogeneous, with hypo-connectivity hypothesised to underlie loosening of associations seen in schizophrenia (Friston and Frith, 1995) and hyper-connectivity thought to lead to increased salience of internal stimuli resulting in delusions and hallucinations (Whitfield-Gabrieli et al., 2009). Such differences in connectivity may also be due to the heterogeneity of the disorder, disease state at the time of the study, medication exposure and also methodological differences between studies (Fornito et al., 2012).

Evidence suggests that there is a link between structural connectivity and functional connectivity (Greicius et al., 2009, Honey et al., 2009, Skudlarski et al., 2008, Goni et al., 2014, Hagmann et al., 2008, Garcés et al., 2016, Chu et al., 2015, Hermundstad et al., 2013).

For example, Marshall et al. (2015) found that occipital cortical oscillations are modulated by individual differences in fronto parietal white matter tracts.

1.2.2.2.1 Task-based Studies of Functional Connectivity in Schizophrenia

Much of the connectivity research in schizophrenia has focussed upon connectivity during task performance such as sensory processing and working memory tasks (Nielsen et al., 2017, Goghari et al., 2017). Whilst results of such studies are heterogeneous, most have shown dysconnectivity; particularly reduced functional connectivity in frontal regions. (Reviewed by Pettersson-Yeo et al. (2011)). Task based functional connectivity studies using MEG have implicated various regions and frequency bands in schizophrenia. For example, a visuo-motor study using amplitude envelope correlations by Brookes et al. (2016) found reduced occipital alpha in patients with schizophrenia. Roiser et al. (2013) also found reduced connectivity during a visual task in schizophrenia but in a fronto-parietal network. They also found that this reduced connectivity predicted lower intelligence. Fujimoto et al. (2013) found reduced connectivity during auditory oddball task. Hirvonen et al. (2017) found reduced phase synchrony in the theta, alpha and gamma bands in patients with chronic schizophrenia during a visual perceptual closure task.

1.2.2.2.2 Resting-State Studies of Functional Connectivity in Schizophrenia

Analysis of spontaneous activity (at rest, when not engaged in a task) is useful in understanding whether any changes represent underlying impairments in the generation of neural activity or whether impairments are only present when performing a task. Resting-state paradigms are also useful in patient populations, as they are not confounded by behavioural performance on a task. In addition, linking with the NMDAR hypothesis of schizophrenia, excitation/inhibition regulates functional connectivity in resting-state networks. For example, a study by Duncan et al. (2013) found a correlation between prefrontal connectivity and prefrontal glutamate in healthy controls. In addition, Stagg et al. (2014) found a correlation between local GABA and motor network functional connectivity at rest. Therefore, for these reasons, resting-state functional connectivity is a useful parameter to explore in patients with schizophrenia.

The majority of studies exploring resting-state connectivity in schizophrenia have used fMRI. Results from ICA-based resting state fMRI studies of schizophrenia are inconsistent whereas the majority of the seed-based resting-state fMRI studies show reduced connectivity in schizophrenia (Yu et al., 2012).

There are a limited number of MEG resting-state studies of schizophrenia and again, methodologies are heterogeneous (Bowyer et al., 2015, Canive et al., 1998, Fehr et al., 2003, Hinkley et al., 2011, Kim et al., 2014, Rutter et al., 2009, Sperling et al., 1998). Only some of these studies have used connectivity metrics in their analysis (Bowyer et al., 2015, Hinkley et al., 2011, Kim et al., 2014). Results of these studies are mixed with one study finding hyper-connectivity (Bowyer et al., 2015), one finding hypo-connectivity (Kim et al., 2014) and another finding both hypo and hyper-connectivity in different brain regions (Hinkley et al., 2011) in schizophrenia. Several studies have used coherence, a frequency domain measure that quantifies coupling in terms of amplitude and phase (Bowyer et al., 2015, Kim et al.,

2014, Hinkley et al., 2011). Using MEG Coherence Source Imaging (CSI MEG) in the 3-50Hz frequency range, Bowyer et al. (2015) found increased amplitude coherence in patients with schizophrenia in the right inferior frontal lobe, left superior frontal lobe, right middle frontal lobe and right cingulate. Kim et al. (2014) found decreased coherence in patients between the PCC and MPFC in the gamma band at rest. They also measured the difference in power spectral density in the Default Mode Network (DMN) between resting and task conditions and found resting DMN activity to be augmented in the PCC in schizophrenia. Using the mean imaginary coherence between a voxel and the rest of the brain, Hinkley et al. (2011) found decreased connectivity in LPFC and right superior temporal cortex and increased connectivity in left extra striate and right inferior PFC in patients with schizophrenia in the alpha band.

1.2.2.2.3 The Impact of Antipsychotic Medication Upon Functional Connectivity

Given the heterogeneity of schizophrenia, it is perhaps unsurprising that results from studies of connectivity in the disorder are also heterogeneous (Pettersson-Yeo et al., 2011). However, this may be due to other factors aside from the disorder itself such as exposure to antipsychotic medication, differences in imaging methodology and analysis technique.

The majority of brain imaging studies of schizophrenia involve patients medicated with antipsychotics. This is because it may be (in certain circumstances) ethically questionable and practically difficult to undertake an imaging study on an untreated patient with schizophrenia. Attempts to elucidate underlying pathophysiological mechanisms of schizophrenia have been made using studies of antipsychotic-naïve first episode patients, patients with prodromal symptoms and first degree relatives. However, when comparing patients with first episode schizophrenia and established schizophrenia, medication exposure continues to be

a confounding factor. Any changes found could be due to the pathophysiology of the disease or due to medication exposure.

Antipsychotics are a heterogeneous group in themselves and are often described as "dirty drugs" as many have affinity for multiple receptors including serotonin, acetylcholine, noradrenaline, histamine and dopamine (Mailman and Murthy, 2010). However, their antipsychotic effect appears to be primarily due to D2 receptor antagonism. Aripiprazole and brexipiprazole (D2 receptor partial agonists) are the only currently available antipsychotics that are not D2 receptor antagonists (Bowles and Levin, 2003). Given that such neurotransmitter systems are widespread throughout the brain, changes in functional connectivity may also depend upon the receptor profile of a particular antipsychotic.

In addition, studies using fMRI are complicated by the fact that the BOLD signal represents neural and vascular processes. Changes in BOLD signal due to antipsychotic medication may be the result of a direct drug action on cerebral blood vessels rather than being solely due to changes in neural activity. For example, D2 blockade reduces dopamine induced vasoconstriction, consequently leading to increased blood flow and increased BOLD signal. Therefore, there may be a direct drug action on cerebral blood vessels rather than the BOLD response solely being due to neural activity (Abbott et al., 2013). In contrast, MEG is a direct measure of neural activity and is therefore, not impacted by effects upon blood flow- however, medication may still act as a confounding factor via a direct effect upon neuronal activity.

Multiple studies have attempted to explore the impact of antipsychotic exposure upon functional connectivity (Stephan et al., 2001, Lui et al., 2010, Klasen et al., 2013, Li et al., 2016). Such studies may help to disentangle the effects of antipsychotics from those of the illness itself. Over recent years, many of these studies have assessed functional connectivity

in schizophrenia pre-and post-treatment with antipsychotics using resting-state paradigms (Lui et al., 2010, Hadley et al., 2014, Sarpal et al., 2015, Kraguljac et al., 2016b, Li et al., 2016). Some have shown normalisation of pre-treatment dysconnectivity following short term treatment (Hadley et al., 2014), whilst others have found incomplete attenuation of dysconnectivity (Kraguljac et al., 2016c). Sarpal et al. (2015) found region specific increases and decreases in connectivity with treatment response (at 12 weeks). A longer term longitudinal study by Li et al. (2016) found a mixed pattern of baseline neural activity abnormalities (measured with amplitude of low frequency fluctuations (ALFF) of BOLD), only some of which normalised after 1 year of antipsychotic treatment.

Some studies have explored the impact of antipsychotics using EEG (Schoen et al., 2011, Takahashi et al., 2010, Kikuchi et al., 2007) but very little work has explored the effects of antipsychotic medications upon neural oscillations or functional connectivity in schizophrenia using MEG. A study by Canive et al. (1998) found reduced alpha peak frequency and power at baseline in patients with schizophrenia. Delta and theta activity were normalised with antipsychotic treatment but no such normalisation of alpha frequency and power was seen.

Overall, due to the heterogeneity of study populations used and differing study designs, there is no clear picture of the impact of antipsychotics upon connectivity in patients with schizophrenia. There appears to be no consistent effect of antipsychotic medication upon fMRI resting-state functional connectivity since some studies find normalisation whilst others do not. It is difficult to draw conclusions regarding their impact upon MEG functional connectivity but future work should take this into consideration either in design or in the drawing of conclusions.

1.3 The Continuum Hypothesis of Schizophrenia

The diagnosis of schizophrenia is categorical, meaning that patients must meet certain criteria to receive a diagnosis (APA, 1994, WHO, 1993). However, the dimensional view of schizophrenia considers the disorder as existing on a continuum (Claridge and Beech, 1995) with sub-clinical psychotic traits in healthy individuals (schizotypy) (Rado, 1953) existing at one end of the spectrum and schizophrenia existing at the other. Authors have argued that schizotypy is "psychopathologically similar but less severe" than schizophrenia (Nelson et al., 2011) or in other words that it is "qualitatively similar but quantitatively different" (Grant, 2015). This dimensional view is supported by studies using factor analysis that find three main dimensions of schizotypy that reflect those of schizophrenia (positive, negative and disorganised) (Bentall et al., 1989). In addition, this continuum model is supported by other research which suggests that not only do schizotypy and schizophrenia share common psychopathology, there is also a genetic and neurobiological overlap (Reviewed by Ettinger et al. (2014)).

One aim of this thesis is to explore the biological validity of the continuum hypothesis of schizophrenia. In addition, I will explore the utility of the study of schizotypy in understanding more about the aetiology and pathophysiology of schizophrenia without the confounder of antipsychotic exposure as previously discussed. What follows is a brief overview of studies exploring the schizotypal spectrum and how these fit with the literature around schizophrenia.

1.3.1 Continuity Between Schizophrenia and Schizotypy

1.3.1.1 Genetic Continuity

Multiple studies using varying methodologies have explored the genetic commonality between schizotypy and schizophrenia. Studies exploring relatives of those with schizophrenia have found that schizotypy is more common in them, suggesting familial correlation between the two (Yaralian et al., 2000, Vollema and Postma, 2002). However, there are very few molecular genetics studies that support a genetic continuity between schizophrenia and schizotypy. Stefanis et al. (2013) found an association between two schizophrenia susceptibility variants and positive schizotypy in a healthy cohort of young men. Using a genome wide association study, Fanous et al. (2007) found an overlap between genetic association profiles for schizophrenia and schizotypy. A recent study showed that various genetic and environmental risk factors for schizophrenia could predict schizotypy, again supporting this notion (Morton et al., 2017). However, Jones et al. (2016) found no association between polygenic risk scores for schizophrenia and psychotic like symptoms in a healthy population. There was a strong association between schizophrenia polygenic risk score and negative symptoms at 16.5 years and also for anxiety disorder at 15.5 years. In addition, a recent study by Hatzimanolis et al. (2017) failed to find an association between polygenic risk scores for schizophrenia and schizotypy scores in a cohort of healthy volunteers.

Overall, these studies do not provide robust evidence for genetic continuity between schizophrenia and schizotypy. However, Grant (2015) argues that healthy individuals with high schizotypy should have less genetic overlap with schizophrenia as this reflects higher resilience. Should they possess more genetic loading for schizophrenia then they would potentially then have developed clinically diagnosable schizophrenia. Alternatively, the lack

of robust evidence for genetic continuity may mean that those with high schizotypy represent a group with purely non-genetic risk.

1.3.1.2 Neurobiological Continuity

1.3.1.2.1 Structural Brain Changes

Grey matter volume reductions and ventricular enlargement are common findings in studies of schizophrenia (Shepherd et al., 2012). In line with such studies, multiple studies have explored structural changes in schizotypy or schizotypal traits with inconsistent results, some showing increased grey matter volume (Nenadic et al., 2015, Modinos et al., 2010), some showing reduced grey matter volume in schizotypy (DeRosse et al., 2015, Ettinger et al., 2012) and others showing region specific changes in both directions (Wang et al., 2015, Wiebels et al., 2016).

1.3.1.2.2 Connectivity in Schizotypy

As discussed, it has been hypothesized that schizophrenia is a syndrome of dysconnectivity (Friston and Frith, 1995, Pettersson-Yeo et al., 2011) and over the last 20 years, this hypothesis has been supported by multiple studies exploring structural and functional connectivity in schizophrenia (Pettersson-Yeo, Allen, Benetti, McGuire, & Mechelli, 2011).

1.3.1.2.2.1 Structural Connectivity

As discussed previously, deficits in structural connectivity have been found in schizophrenia that suggest a progressive decline throughout the disorder. Studies have explored structural connectivity in schizotypy with mixed results, some finding decreased FA (Nelson et al.,

2011), some finding increased FA (Smallman et al., 2014) and others finding both increased and decreased FA depending upon brain region (Volpe et al., 2008).

1.3.1.2.2.2 Functional connectivity

As with schizophrenia, much of the resting-state functional connectivity research in schizophrenia continuum disorders has utilised fMRI. The most recent study by Zhu et al. (2017) found reduced functional connectivity between bilateral precuneus and contralateral parahippocampal gyrus in schizotypal personality disorder and a negative correlation between functional connectivity and total SPQ score. Other studies have found mixed patterns of dysconnectivity in schizophrenia continuum disorders with region dependent increases and decreases in connectivity (Wang et al., 2015, Zhang et al., 2014b, Lagioia et al., 2010). To date, there are no published resting-state MEG studies of schizotypy.

Other studies of functional connectivity in schizotypy have used task based approaches. For example, using a self-reflection fMRI task in adolescents, Debbane et al. (2014) found that positive schizotypy correlated with activation of the dorsomedial prefrontal cortex (dmPFC) and the posterior cingulate cortex (PCC), as well as the dorsolateral PFC and the lingual gyrus. This reflects similar findings in patients with schizophrenia (van der Meer et al., 2010).

1.3.2 Utility of Using Schizotypy to Understand Schizophrenia

Whilst not entirely consistent, these findings suggest that there may be neurobiological continuity between schizophrenia and schizotypy, particularly when exploring specific dimensions of schizotypy. If we consider this to be the case, schizotypy in the general population may be a valuable resource in understanding more about schizophrenia. For

example, we may be able to gain valuable insights into the underlying aetiology and neurobiology of schizophrenia in an un-medicated group, free from the potential confound of medication. We may also understand more about protective factors that have prevented healthy individuals with subclinical traits from developing schizophrenia.

1.4 Ketamine as a Model of Schizophrenia

As previously discussed, it has been postulated that NMDAR antagonists such as Ketamine model schizophrenia (Frohlich and Van Horn, 2014). This is in part due to the finding that such drugs exacerbate symptoms in patients with schizophrenia (Lahti et al., 1995) and mimic positive, negative and cognitive symptoms of schizophrenia in healthy controls (Adler et al., 1999, Krystal et al., 2005). In addition, the NMDAR has been implicated in schizophrenia through molecular studies of dysbindin (a protein linked to NMDAR function and working memory (Karlsgodt et al., 2011). Genes regulating the NMDAR co-agonist D-serine have also been found to be disturbed in schizophrenia (Chumakov et al., 2002). SPECT studies have also found in vivo evidence of NMDAR hypofunction in schizophrenia (Pilowsky et al., 2006). Thus molecular, genetic and imaging studies support the use of ketamine as a model of schizophrenia.

In addition, again, as previously discussed, it is postulated that symptoms of schizophrenia result from dysconnectivity and this is supported by multiple studies (Fornito et al., 2012). Studies suggest that synchronised oscillatory activity is dependent upon the interaction between excitatory (glutamatergic) and inhibitory (GABAergic interneuron) activity (Uhlhaas et al., 2008). Given that such transmitter systems are perturbed with ketamine and that dysconnectivity has been implicated in schizophrenia, we may expect the administration of ketamine, a model of schizophrenia, to result in dysconnectivity in healthy individuals.

Several studies of the impact of ketamine upon functional connectivity in healthy controls have found increased connectivity following ketamine administration (Hoflich et al., 2015, Rivolta et al., 2015, Driesen et al., 2013a, Anticevic et al., 2015a). Of these studies, the majority used fMRI to explore functional connectivity, aside from resting-state MEG studies by Rivolta et al. (2015) and Muthukumaraswamy et al. (2015).

Using transfer entropy, Rivolta et al. (2015) found thalamo-cortical hyper-connectivity involving the visual cortex. Other studies have found reduced connectivity following ketamine administration (Kraguljac et al., 2016a, Scheidegger et al., 2012). Using ICA in MEG, Muthukumaraswamy et al. (2015) also found reduced fronto-parietal network activity following ketamine administration. Interestingly, Driesen et al. (2013b) found decreased connectivity during a working memory task but increased connectivity at rest (Driesen et al., 2013a) following ketamine. This suggests that its effects upon functional connectivity may be state dependent. Effects may also relate to the amount or duration of ketamine use with a more naturalistic study finding reduced resting-state functional connectivity in chronic ketamine users (Liao et al., 2016).

When considering the link between the neurological impact of ketamine and the neuropathology of schizophrenia, Anticevic et al. (2015a) found increased connectivity following ketamine administration and in a group of patients with early schizophrenia but reduced functional connectivity in chronic schizophrenia. The authors therefore suggest that ketamine is a better model for the early stages of schizophrenia than the late stages.

This is supported by studies such as Liao et al. (2016) who found hypo-connectivity in chronic ketamine users suggesting acute versus chronic NMDAR antagonism may have differential effects upon functional connectivity. Animal studies also support this, with a

study by Ahnaou et al. (2017) finding increased phase amplitude coupling following acute administration of ketamine but decreased coherence with chronic ketamine administration.

The neurochemical effects of ketamine are also reflected in those of schizophrenia, particularly early stages. For example, Stone et al. (2012) found increased glutamate in the anterior cingulate cortex following ketamine administration, in line with MRS studies of first episode schizophrenia finding increased glutamate (Theberge et al., 2002) in patients.

Given this evidence, the administration of ketamine may be a useful way of indirectly exploring the neurobiology of schizophrenia without confounding factors such as medication exposure.

1.5 Objectives

Together, the dysconnectivity hypothesis of schizophrenia and the NMDAR hypothesis can explain some of the psychopathology of the disorder. However, studies of functional connectivity and in vivo neurochemistry in schizophrenia are inconsistent. This thesis seeks to explore these hypotheses further in multiple groups of patients with schizophrenia using novel neuroimaging analysis methods. The first experimental chapter explores functional connectivity using both MEG and FMRI in two groups of patients with schizophrenia. The second explores functional connectivity in the context of the continuum hypothesis of schizophrenia in healthy participants with high and low schizotypy scores. The third experimental chapter attempts to elucidate functional connectivity findings in schizophrenia further by exploring the impact of disease stage. The fourth looks at functional connectivity following ketamine exposure in order to explore this drug as a model of schizophrenia. The fifth experimental chapter combines together the results from all of these studies using a meta-analytic approach in order to strengthen conclusions. Finally, the sixth experimental

chapter uses MRS to look at GABA and this is explored this in the context of functional connectivity.

Given the vast amounts of heterogeneous findings in the literature, the overall aim of the thesis is to use robust and standardised analysis pipelines and metrics of functional connectivity, in multiple cohorts of participants, to explore the neurobiology of schizophrenia, the schizophrenia spectrum and a pharmacological model of schizophrenia. In particular, the thesis will focus on functional connectivity measured with MEG. As previously discussed, there are many ways of exploring MEG functional connectivity but for consistency and due to the fact that it is a robust and repeatable measure (Colclough et al., 2016), I have chosen to use amplitude-amplitude coupling in classic oscillatory bands throughout this thesis. Also, although as discussed, several neurotransmitters are implicated in the pathogenesis of schizophrenia, given our previous work on GABA, we have chosen to focus upon this neurotransmitter.

Chapter 2 Methods

2.1 Imaging Methods

2.1.1 MEG

Magnetoencephalography (MEG) is a non-invasive functional brain imaging technique used to measure synchronised neuronal activity. It measures weak magnetic fields, generated by the electrical activity of many thousands of neurones and is therefore a direct measure of neural activity. In addition, it also has the advantage over other imaging techniques of having a high temporal resolution (within the millisecond range) (Baillet et al., 2001).

2.1.1.1 MEG Signal Generation and Detection

The electrical current created by post-synaptic potentials in dendrites of neurones generates associated perpendicular magnetic fields. These magnetic fields are small, much smaller than environmental magnetic fields generated by vehicle traffic for example. Therefore, only when multiple (thousands to millions) of aligned neurones fire synchronously is this magnetic field large enough to be detected by the MEG system (Singh, 2006). The system can only detect magnetic fields from sources orientated perpendicular to the brain surface. However, given the complex structure of the cortex, it is likely that the proportion of grey matter aligned radially and therefore not leading to a magnetic field that can be detected is small (Hillebrand and Barnes, 2002).

For the MEG system to detect a magnetic field, several requirements must be met:

1. The population of neurones must be large and firing synchronously.
2. The population of neurones/dendrites must all be aligned in the same direction.

3. This alignment must be perpendicular to the surface of the brain. e.g. apical dendrites of pyramidal cells

In order to detect these small neuro-magnetic fields, the MEG system is extremely sensitive. The system houses 200-300 Superconducting Quantum Interference Devices (SQUIDs) that are distributed evenly over the head in a liquid helium dewar reservoir that maintains them at -269°C. SQUIDs are small rings of superconducting material with insulating breaks. Each SQUID is coupled to a magnetometer, which is a small loop that picks up magnetic activity and magnifies it in order for the SQUID to sense it. The breaks within SQUIDs have a superconducting effect and when a magnetic field passes over it, this causes a potential, which fluctuates according to how much magnetic field is passing over the loop.

Given the sensitivity of the system in detecting very weak magnetic fields, it is important that environmental magnetic activity (or noise) is minimised as much as possible. This is done in two ways:

1. Shielding: The MEG system is housed in a magnetically shielded room (MSR), built of multiple layers of aluminium and mu metal. This helps to block out external environmental magnetic fields.
2. Sensor design: Specially designed magnetometers called gradiometers help to discriminate neural signals from noise. There are two different types of gradiometers (named according to their design); axial and planar. Both types of gradiometers are made of oppositely wound coils. Magnetic fields decay rapidly the further from the source they become and gradiometers are therefore more sensitive to closer neural signals rather than more distant environmental or physiological sources. Given that

the magnetic fields deteriorate with distance, MEG can only be used for deep sources provided the signal to noise ratio is high enough (Vrba and Robinson, 2001).

The system used in all subsequent studies discussed in this thesis is a CTF 275 axial gradiometer design. Third-order gradiometer mode is constructed using reference magnetometers midway up the dewar. This gives enhanced noise rejection without significantly reducing depth sensitivity. All data in this thesis was processed using third-order mode.

2.1.1.2 MEG Analysis

There is an increasing realisation within the neuroscience research field that neural oscillations play a critical role in perception and cognition and MEG is well-suited to studying these oscillations (Donner and Siegel, 2011). Oscillations are waves of complex neural activity that can be described according to their phase, amplitude and frequency. They can be divided into multiple frequency bands though spectral decomposition. Each frequency within an oscillation can be represented using the power spectrum. Through spectral analysis, the signal is expressed as a function of frequency rather than time, a transformation known as the Fourier transform. Given that amplitude and therefore power varies over time, the signal can be divided into shorter segments. Spectral analysis can then be carried out on these segments to obtain a more dynamic assessment of activity, known as time-frequency analysis. Using the Hilbert transform, time-varying amplitude envelopes can be obtained which can then be used to generate time/frequency/amplitude spectrograms of data (Brookes et al., 2011, Swettenham et al., 2009).

In addition to understanding the neural signal in "sensor space", we are also interested in understanding the signal in "source space", i.e., where it has come from in the brain. In order to localise the source of measured neural activity, we must solve the "inverse problem". Working out what magnetic field would be measured in a detector array given the magnitude, position and orientation of a neuronal current source (solving the forward problem) is relatively straight forward (Singh, 2006). However, solving the inverse problem, or localising a current generator given an externally measured magnetic field is more difficult due to the infinite number of possible solutions. This is known as non-uniqueness (Mosher et al., 1999). Through adding additional information (or a priori constraints), the possible solutions can be reduced to one unique solution. There are multiple ways to solve the inverse problem, including equivalent dipole fitting and distributed current models. Equivalent dipole fitting models a single active source at any given moment (Wood, 1982) whilst distributed current models, such as minimum norm, estimate a continuous current distribution that is generating the observed response (Hamalainen and Ilmoniemi, 1994). Another commonly used method is a type of spatial filtering called beamforming (Hillebrand et al., 2005). This involves using weighted sums of MEG detector outputs (and the forward model as a prior) to estimate the "virtual electrode output" within the brain. Throughout this thesis, a linearly constrained minimum variance (LCMV) beamformer has been used (Van Veen et al., 1997). The use of a beamforming approach is advantageous, particularly when analysing resting-state data, as it can be used to study activity that is not tightly phase-locked to a stimulus (Singh, 2006). In addition, it offers additional noise rejection that is beneficial when analysing non-averaged data (Vrba, 2002).

2.1.1.3 MEG Functional Connectivity Analysis

The high temporal resolution of MEG allows the identification of precise timings of activity within the brain. Temporal relationships in activity between brain areas can then be identified using this information. This is known as functional connectivity. There are multiple methods of assessing functional connectivity using MEG (Siegel et al., 2012) including phase-phase, phase-amplitude and amplitude-amplitude coupling. Figure 2.1 shows a schematic of phase coherence (phase-phase coupling) and amplitude correlation (amplitude-amplitude coupling).

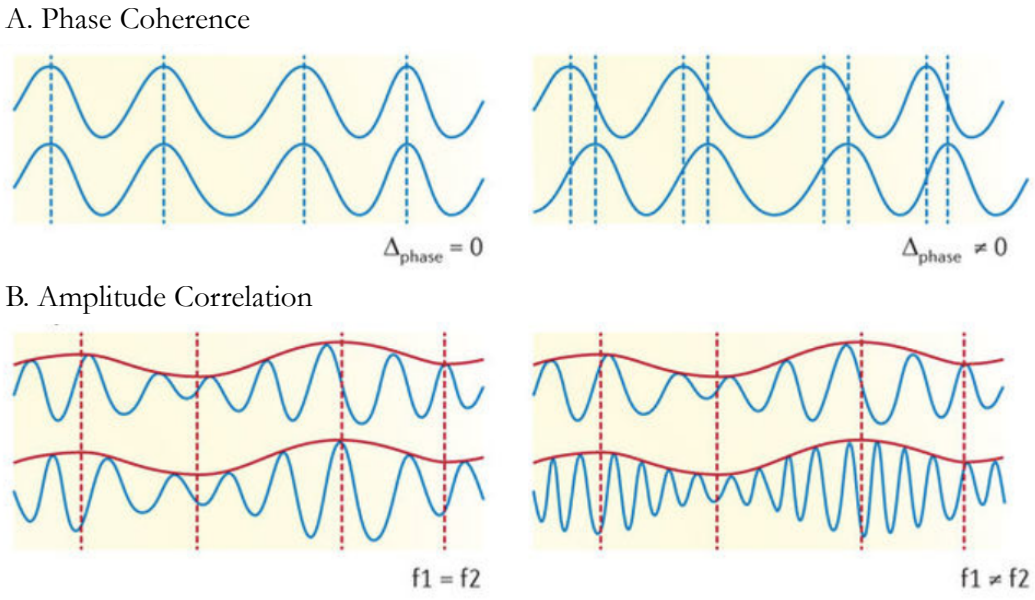


Figure 2.1: Diagram of phase coherence and amplitude correlation (Adapted from Siegel et al., 2012)

A. Phase coherence quantifies the consistency of the phase between two simultaneous signals with the same frequency. Left panel shows phase coherence with zero phase lag. Right panel shows phase coherence with non-zero phase lag.
B. Amplitude correlation assesses the synchronisation of power sources from different sources. Signals can have the same (left panel) or different (right panel) underlying frequencies.

Spatial reduction to analyse correlation then usually involves a seed based, ICA (Independent Component Analysis) or atlas-based approach. In seed-based approaches, a virtual sensor within the brain is chosen and correlations are explored between the seed and other brain regions (Hipp et al., 2012). ICA based analysis is a data-driven approach whereby patterns of activity are explored across the whole brain, revealing networks of synchronised activity over time (Brookes et al., 2011). Finally, atlas-based approaches involve dividing the brain into pre-defined regions and comparing activity across all regions.

Over recent years, amplitude envelope correlation has been used extensively (Brookes et al., 2016, O'Neill et al., 2015) and has been found to be robust and repeatable (Colclough et al., 2016) and successful in identifying differences between patients with schizophrenia and healthy controls (Brookes et al., 2016). I have therefore chosen to use this method throughout this thesis. Figure 2.2 outlines the methodology. In summary, measured data is frequency filtered into frequency bands of interest. Following this a beamformer is used to reconstruct time-courses in the areas of interest. In this thesis, brain regions have been defined using the Automated Anatomical Labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002) which includes 90 sources of interest in the cortex and subcortical regions. Given the potential for signal leakage and artefactual correlation, a method to reduce this must be used. I have chosen to orthogonalise signals using multivariate linear regression to reduce zero-time lag correlation for any pair of brain regions within the AAL atlas (Colclough et al., 2015). From here, for every frequency band and AAL region, the Hilbert envelope (amplitude envelope) of source-space neural oscillatory activity are then computed from orthogonalised signals. Further analysis then involves looking at the correlation between amplitude envelopes in one AAL region and another, thus providing a measure of connectivity between brain regions.

Prior to further statistical analysis, a variance stabilising transformation, (Fishers Z transformation) is then applied to the correlation coefficients to normalise their behaviour and reduce estimation bias when averaging (Silver and Dunlap, 1987). The theoretical variance of the z statistic is then calculated in order to transform Fishers Z scores into z statistics. This corrects for variable number of trials (and hence degrees of freedom) for each person. Connectivity matrices can then be constructed using z statistics as edge weights for all AAL atlas regions (Figure 2.3).

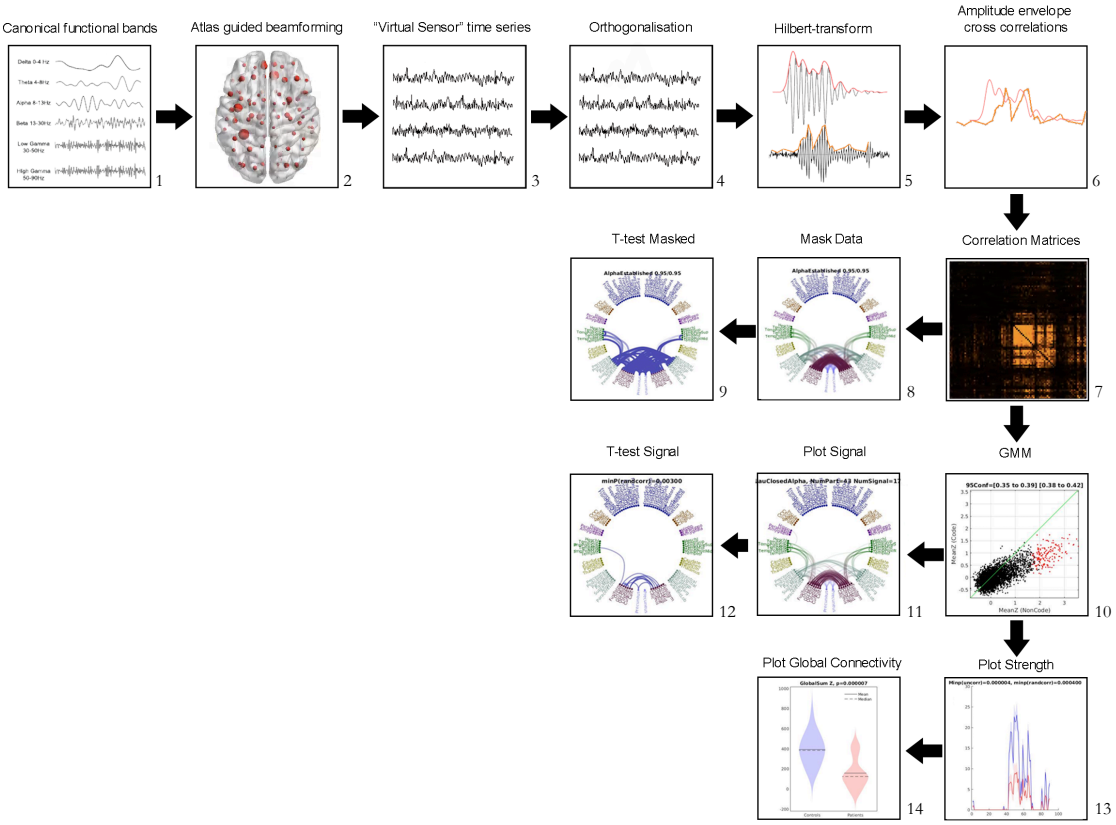


Fig 2.2 MEG analysis pipeline for resting-state functional connectivity.
1-7. We used an AAL atlas-guided beamforming approach to define 90 cortical and subcortical seed regions. We first filtered data into canonical functional bands and then reconstructed virtual sensor time series in each of the 90 AAL regions. The data was orthogonalised to suppress source leakage. We applied a Hilbert transform to give estimates of amplitude and phase for each of these regions. Amplitude envelopes were then cross-correlated to derive the degree of connectivity between AAL regions. z statistics were derived from the correlation coefficients and used to construct connectivity matrices.
8-9. Data was initially masked to show only the highest 5% of connections and this was followed by a t-test of these connections.
10-12. A Gaussian mixture modelling approach was also used to define connections as signal or noise. "Signal" connections were plotted onto connectivity maps, followed by t-tests of these connections. (Blue=lower connectivity in these connections in cases.)
13. Strength of connectivity was calculated by summing z statistics of signal connections horizontally. (Red=Cases. Blue=Controls)
14. Global connectivity was calculated by summing all z statistics of signal connections. Mean, median and t-test. (Red=Cases. Blue=Controls)

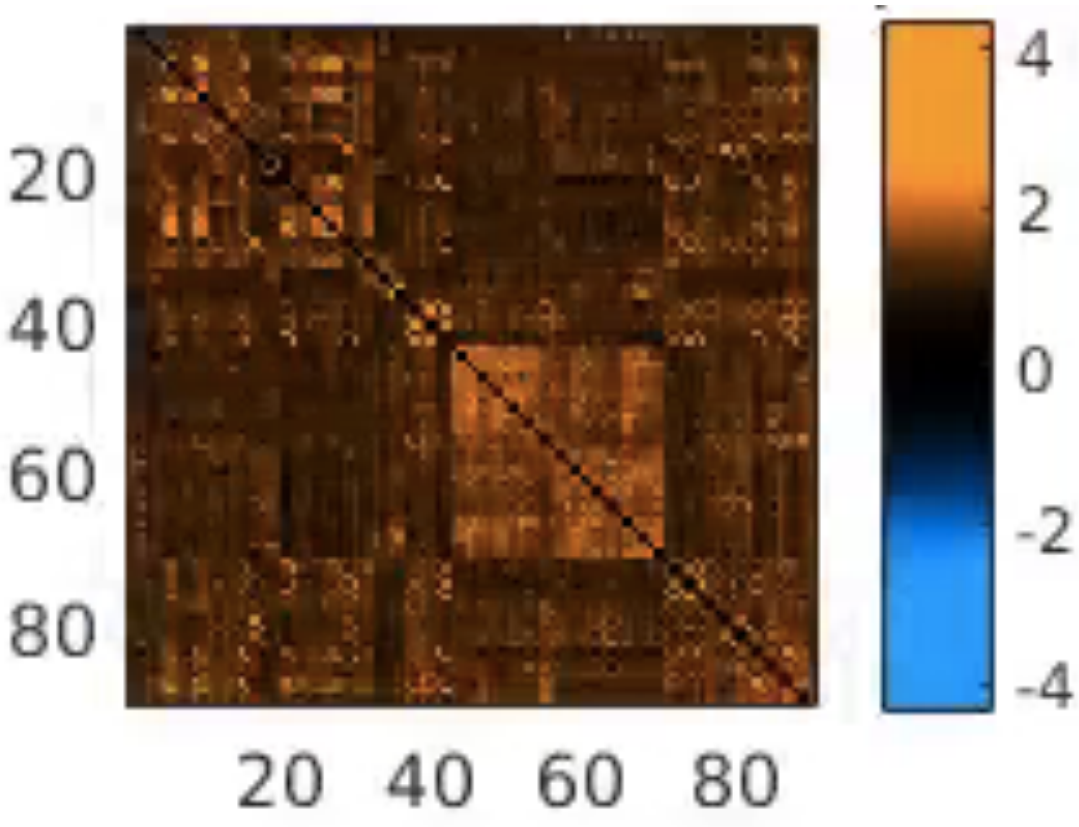


Fig 2.3 Correlation Matrix.
Correlation matrix showing amount of correlation between AAL nodes. x and y axes represent AAL nodes from 1-90. Orange points= Positive correlation between regions. Black points=No correlation between regions. Blue points=negative correlation between regions.

From here further statistical analysis can be used. One such statistical analysis approach used throughout this thesis involves classifying connections into "signal" or "noise" using a statistical thresholding procedure based on Gaussian mixture modelling (GMM) (Plataniotis and Hatzinakos, 2000). Using the Expectation-Maximisation algorithm with two components randomly selected as the initial component means, a Gaussian mixture distribution is fitted to z statistics by maximum likelihood. In each frequency band, only connections with a greater than 50% probability of being within the signal are then accepted as valid. The noise distribution is modelled separately for each cohort and z-scores are corrected for this noise model. This analytical step is important as it corrects for any general bias in signal to noise ratio (SNR) between two cohorts. General SNR is therefore normalised

by this procedure and consequently any remaining statistical differences between groups are more likely to be due to true group differences. In addition, the method allows for statistical thresholding because connections can be labelled as "signal", rather than "noise" and only these are tested for statistical differences. See Appendix 1 for further details regarding the benefits of utilising a GMM approach.

Throughout this thesis, connectivity is displayed on circular connectivity maps to show connections between AAL nodes. An example of this with explanations can be seen in Figure 2.4. Figures in this thesis show connectivity maps without AAL labels.

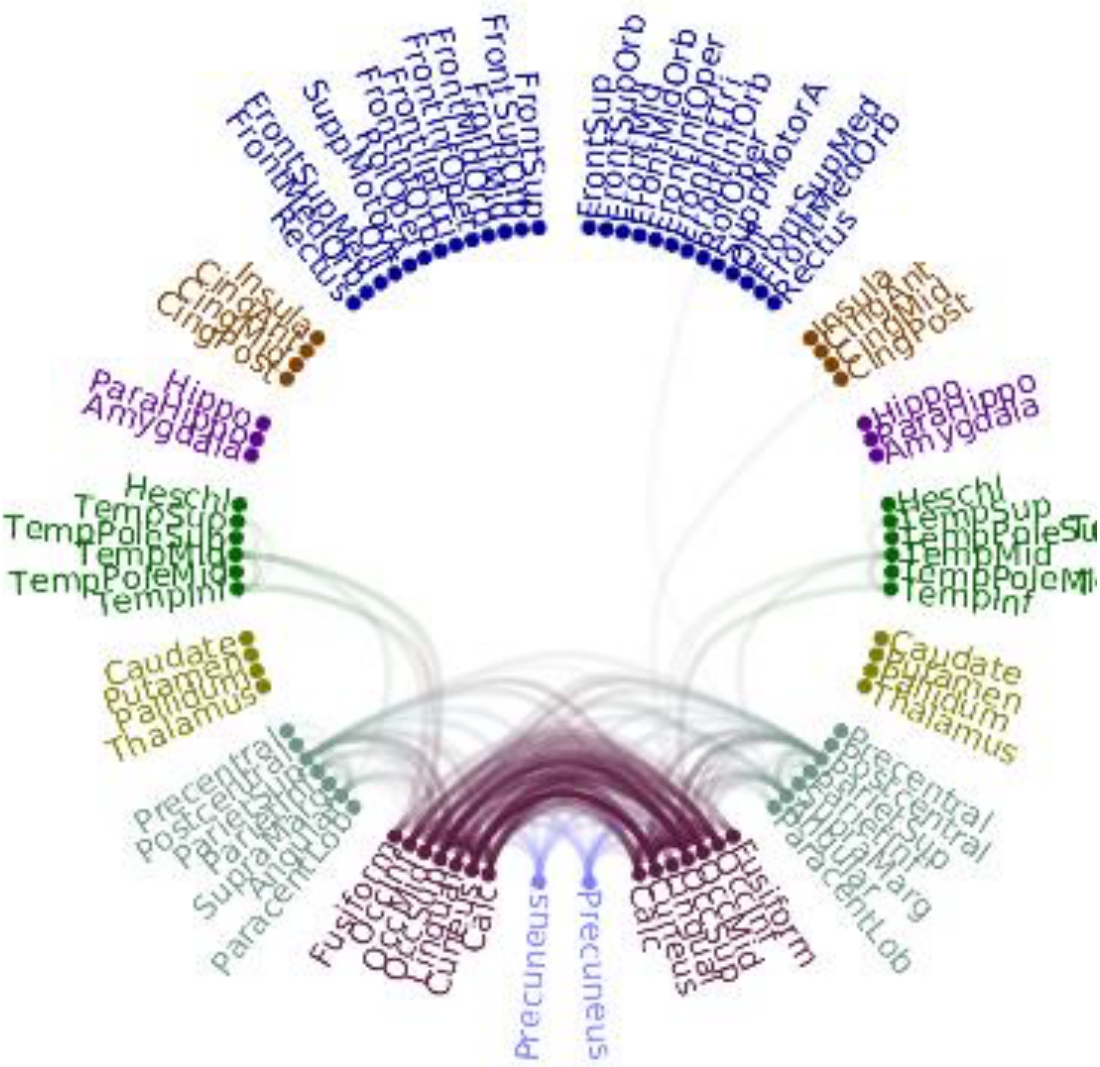


Fig 2.4 Circular Connectivity Map.
Connectivity map showing connections between AAL nodes. The circle is anatomically organised with the right hemisphere on the right side and the left hemisphere on the left. Labels show AAL regions, starting from the top with frontal cortex, cingulate cortex, hippocampus and amygdala, temporal cortex, deeper brain regions, parietal cortex, occipital cortex and precuneus. Lines represent connections between these regions. Darker lines represent stronger connections and paler lines represent weaker connections. The colour represents the region of the connection in this instance. When the map is showing differences in connectivity between groups, blue lines represent reduced connectivity and red lines represent increased connectivity.

2.1.2 Magnetic Resonance Imaging (MRI)

MRI is a non-invasive imaging technique that allows the direct visualisation of soft tissues as well as giving information regarding function. It exploits the fact that atomic nuclei absorb and emit energy when placed in an external magnetic field. Given that hydrogen atoms exist in abundance in the human body, (particularly in fat and water), these are often used to generate the radio frequency signals that are detected by MRI systems.

Hydrogen nuclei possess an intrinsic property called nuclear spin, meaning that the nuclei rotate. To a degree, when placed in a magnetic field, the nuclei have a tendency to align themselves parallel with the field. The overall direction of many nuclei when placed in a magnetic field is called the net magnetisation. This net magnetisation is proportional to the magnetic field with larger fields producing greater alignment. Those protons aligned with the magnetic field are in a lower energy state and those not aligned are in a high-energy state.

Spinning protons precess or wobble around the axis of the external magnetic field. The frequency of this precession or resonance frequency is known as the Larmor frequency and is proportional to the strength of the magnetic field.

When an electromagnetic radio frequency is applied at the same frequency as the resonance frequency of the proton, they absorb energy and the proton moves to a higher energy state. Net magnetisation is then brought away from equilibrium, which results in the nuclei precessing around the field with a frequency of 42.58 MHz/T (De Graaf, 2013). This results in radio waves at this frequency being emitted from the body, which can then be measured and analysed. The energy is then retransmitted when the radio frequency transmission (or RF pulse) is turned off. Protons then return to their original orientation of external magnetic field. This is known as T1 recovery to thermal equilibrium. This recovery rate is different for every tissue and hence allows different tissues to be distinguished. At the same time that the

protons are returning back to align along the field, individual protons start to interact with each other, leading to de-phasing of the spins, which reduces the measured signal. This is known as T2 relaxation and its rate is also dependent on the tissue types within the region. Signals are received and measured by a receiver coil and MR sequences then distinguish tissues based upon their T1 and T2 decay processes. Finally, certain substances are magnetic in nature and directly lead to inhomogeneities in the primary magnetic field –the so-called susceptibility artefact. This variation in magnetic field leads to additional dephasing as different spins see a different magnetic field and hence precess at slightly different rates. This dephasing is known as T2* relaxation, and is particularly useful for fMRI (Filippi, 2009).

2.1.2.1 Functional Magnetic Resonance Imaging (fMRI)

fMRI is a method of measuring functional activity and connectivity using MRI. The technique detects changes in blood flow, known as the Blood Oxygen Level Dependent (BOLD) signal (Kwong et al., 1992). It is therefore an indirect measure of neural activity and assumes a correlation between neural activity and blood flow. This is known as neurovascular coupling and suggests that neuronal function results in increased blood flow and therefore increased oxygenated haemoglobin (oxy-haemoglobin) concentration in activated regions of the brain. Oxygenated and deoxygenated haemoglobin have different magnetic properties and the method takes advantage of this. Oxy-haemoglobin is diamagnetic and deoxy-haemoglobin is paramagnetic, leading to faster T2* relaxation. More oxygenation therefore results in a higher signal and a brighter image (Amaro and Barker, 2006). Neural activity therefore results in a transient increase in MR signal, known as the BOLD haemodynamic response function (HRF).

During an fMRI study, an activation map is produced which depicts the level of engagement of particular brain regions during a task or in response to a stimulus. The scale of such responses can then be compared between conditions or between subjects. This data can then be used to construct maps representing regions of the brain activated by specific tasks by superimposing signal onto structural images of the brain.

There are several important considerations when evaluating fMRI. Whilst BOLD has excellent spatial resolution, it has relatively poor temporal resolution of 1-2 seconds. This is because, following stimulus onset, it takes several seconds for HRF to peak and several seconds to return to baseline following stimulus cessation. In addition, physiological noise (including the cardiac and respiratory cycles) can produce BOLD artefacts (Birn et al., 2006, Dagli et al., 1999). Such effects may lead to confounding in case-control studies where factors such as medication exposure and anxiety for example (which could affect this physiological noise) may have a differential effect upon cases and controls (Murphy et al., 2013).

2.1.2.1.1 fMRI Resting-State Connectivity Analysis

At rest, fMRI detects low frequency (~0.01-0.1Hz) BOLD oscillations. Functional connectivity analysis in fMRI involves estimating the correlation in activity of BOLD time-courses in distinct brain regions. There are multiple methods for exploring this but the most popular include seed based correlation analysis (Biswal et al., 1995) and independent component analysis (ICA) (Rogers et al., 2007). Seed-based analysis refers to a method whereby correlation in BOLD time-series is assessed between a seed region and other brain regions. ICA involves the data-driven identification of patterns of BOLD activity across the whole brain, resulting in networks of activity. Alternatively, atlas-based approaches, similar to those described previously for MEG analysis, can be used. Using this method, the brain

is parcelled into regions, using an atlas such as the AAL atlas, in order to reduce the data from hundreds of thousands of voxels to hundreds of regions. BOLD time-series can then be correlated between all regions of the atlas (Faria et al., 2012).

In this thesis, for consistency, I have chosen to use AAL-atlas based functional connectivity analysis similar to that used for MEG analysis.

2.1.2.2 Magnetic Resonance Spectroscopy (MRS)

MRS is an MRI method involved in the non-invasive detection and quantification of brain metabolites in vivo. The technique takes advantage of the fact that each metabolite produces a unique MRS signal because protons within the molecule experience different spin frequencies or resonance frequencies (De Graaf, 2013). As previously discussed, atomic nuclei such as the hydrogen nucleus (^{1}H) exhibit resonance behaviour in a magnetic field. However, within different metabolites, this resonance frequency or Larmor frequency is not uniform but is dependent upon the chemical environment of the molecule. This variation in resonance frequency is due to concepts known as chemical shift and J-Coupling.

Resonance frequency is partially determined by the external magnetic field to which a proton is exposed, however, within a metabolite, the nucleus is shielded from the external magnetic field by surrounding electrons. Increased shielding occurs when an electron cloud is drawn closer to a hydrogen proton and results in reduced resonance frequency (and the reverse is also true). This is known as chemical shift.

When there is more than one type of chemical environment for hydrogen protons within a molecule (e.g. CH_3), J-coupling occurs. This is the correlation of different nuclei in the same

molecule mediated through their binding electrons. Since each metabolite has a different chemical structure, J-Coupling helps to distinguish between them.

MRS is able to detect multiple brain metabolites, providing their concentration within the brain is sufficient for detection. Water is the most abundant proton containing brain metabolite and therefore contributes most to the signal detected by MRS. Water suppression pulses are therefore used to suppress the water peak to allow visualisation of spectra from other, much less abundant metabolites. Water is suppressed by selectively exciting protons that constitute the water peak by applying an RF pulse with the same frequency as water.

Usually, MRS is used to detect and quantify metabolites within a specified region of interest within the brain, known as single voxel MRS. Again, through the use of RF pulses, slices in x, y and z orientations are selected, the intersection of which creates the voxel in which metabolites are quantified.

The detected signal is then plotted onto a spectrum where the x axis represents frequency and the y axis represents amplitude of the signal. An example MRS spectrum can be seen in Figure 2.5. Metabolite concentration can then be calculated by measuring the area under its peak (Stagg and Rothman, 2014).

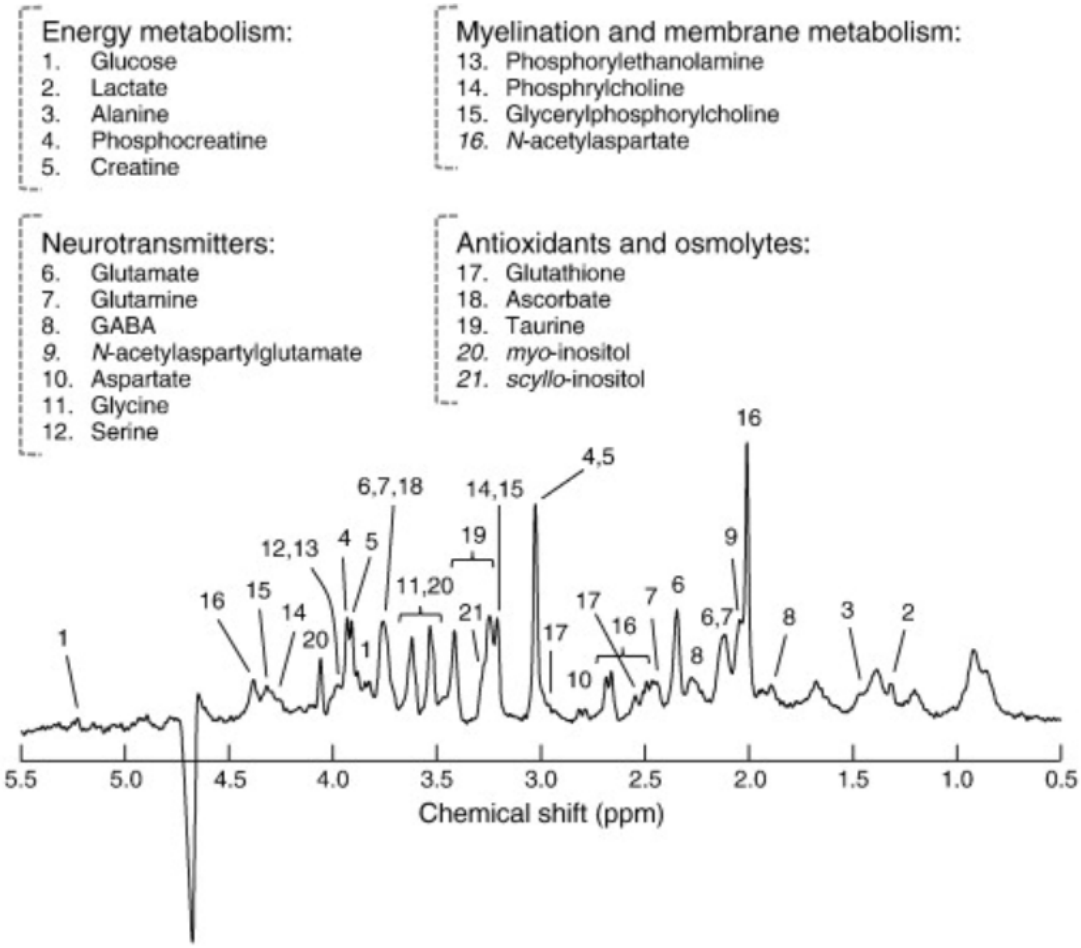


Figure 2.5 Example 1H MRS Spectrum
Reproduced from Duarte et al. (2012)
Example 1H MRS spectrum acquired at 14.1T in the rat hippocampus. Spectrum indicates individual metabolites and their role in brain physiology.

2.1.2.2.1 GABA MRS

The quantification of GABA is technically difficult for several reasons. Firstly, GABA is found at relatively low concentrations in the brain (~1-2mM) (Table 2.1)(Duarte et al., 2012). In addition, as depicted in Figure 2.5, the GABA peaks are obscured by the peaks of several other more abundant metabolites, in particular, the 3.0ppm Cr peak. Therefore, in order to quantify GABA, a technique called J-editing is used (Mullins et al., 2014). This technique

uses the physical properties of the GABA molecule to separate it from the other metabolites in the spectre. The GABA molecule has a weakly coupled spin system. This refers to coupling that occurs when adjacent spins within a molecule affect the field experienced by a spin. As a result, the GABA spectrum contains multiplets, or signals that are split into sub-peaks. GABA contains three methylene groups which results in three distinct multiplets in the spectrum occurring at 1.9, 2.3 and 3.0ppm. The GABA signal at 3.0ppm is coupled to a signal at 1.9ppm. When a frequency-selective pulse (editing pulse) is applied at 1.9ppm, coupling of the signals means that there will be an effect at both 1.9 and 3.0ppm, leaving uncoupled resonances unaffected. This is referred to the "ON" scan. The scan is then repeated without the editing pulse. This is known as the "OFF" scan and the difference between the spectre obtained during repeated ON and OFF scans results in a spectrum only containing signals affected by the pulse i.e. the GABA spectrum. This is known as J-difference editing and is used in MEGA-PRESS, the method used in this thesis (Mullins et al., 2014). GABA is then quantified by measuring the area under the peak, modelled as a Gaussian.

Table 2.1 Brain metabolite concentrations (mM). Adapted from Duarte et al. (2012)

Alanine	0.3
Ascorbate	1.4
Aspartate	2.1-3.1
Creatine	3.2-5.8
Phosphocreatine	2.2-4.5
GABA	1.3-2.5
Glutamine	1.6-2.2
Glutamate	8.9-12.8
Glutathione	1.1-1.4
Glycine	1.2
Glucose	1.4-2.2
Myo-inositol	4.9-5.7
Scyllo-inositol	0.3-0.4
Lactate	0.5-0.7
N-Acetylaspartate	11-13.5

However, the signal detected in an MRI scanner cannot be used to quantify a metabolite and must be calibrated, usually by referencing to another internal reference compound, such as water or creatine. Creatine (Cr) is often used for this purpose by calculating the ratio of GABA to Cr. Since Cr is measured in the same spectrum as the other metabolites, this prevents errors from partial volume effects within the voxel and regional magnetic field susceptibility variations (Li et al., 2003).

However, referencing to Cr may act as a confounder since some studies in schizophrenia have found a difference in Cr between patients and controls (Ongur et al., 2009). This is not a consistent finding, however as others have found no difference in Cr between patients and controls (Marsman et al., 2014). Alternatively, water can be used as a reference by completing another scan without the usual water suppression. The water peak has a higher SNR and is easier to model, however, GABA/H2O quantification must be corrected for the

composition of the tissue (i.e. the relative amounts of grey matter, white matter and CSF) (Mullins et al., 2014) due to the differing levels of water in these tissues. Studies of MRS GABA in schizophrenia have used both Cr and water as a reference and there is no clear consensus as to which reference compound is best. In this thesis, I have therefore chosen to quantify both and compare the two, since for a patient effect to be truly robust, I would suggest that it must be present in both GABA/Cr and GABA/H2O measures.

Chapter 3 A Multimodal Study of Resting-State Connectivity in Schizophrenia

3.1 Rationale

Multiple studies report functional dysconnectivity in schizophrenia. (Reviewed by Pettersson-Yeo et al. (2011)). However, very few studies have explored resting-state connectivity in schizophrenia using MEG and results are inconsistent (Bowyer et al., 2015, Kim et al., 2014). In addition, to date, only one study directly compares resting-state connectivity using both fMRI and MEG in the same sample (Houck et al., 2017). In this study, I sought to elucidate the relationship between schizophrenia and resting-state connectivity using MEG in two separate samples of individuals with schizophrenia. I also sought to compare resting-state connectivity using fMRI and MEG in one of these cohorts.

3.2 Background

It has been hypothesized that schizophrenia is a syndrome of dysconnectivity; referring to abnormal integration both within and between brain regions (Friston and Frith, 1995, Stephan et al., 2006, Stephan et al., 2009a). Over the last 20 years, this hypothesis has been supported by multiple studies exploring functional connectivity in schizophrenia (Pettersson-Yeo et al., 2011), specifically, the correlation in neural activity in different brain regions over time. Functional connectivity can be measured in vivo using multiple neuroimaging techniques such as fMRI, EEG and MEG. Results of such studies are heterogeneous and it is hypothesised that hypo-connectivity may underlie loosening of associations seen in schizophrenia (Friston and Frith, 1995) and hyper-connectivity may lead to increased salience of internal stimuli resulting in delusions and hallucinations (Whitfield-Gabrieli et al., 2009).

Such differences in connectivity may also be due to the heterogeneity of the disorder, disease state at the time of the study, medication exposure and also methodological differences between studies (Fornito et al., 2012).

Much of the connectivity research in schizophrenia has focussed upon connectivity during task performance such as sensory processing and working memory tasks (Nielsen et al., 2017, Goghari et al., 2017). Analysis of spontaneous activity (at rest, when not engaged in a task) is useful in understanding whether any changes represent underlying impairments in the generation of neural activity or whether impairments are only present when performing a task. In addition, resting-state paradigms are useful in patient populations, (where there may be significant impairment such as cognitive dysfunction), as they are not confounded by behavioural performance on a task (Fox and Greicius, 2010).

The majority of studies exploring resting-state connectivity in schizophrenia have used fMRI. fMRI exploits the blood oxygen dependent (BOLD) signal which is thought to be a correlate of neural activity (Buxton, 2002). The relationship between neural activity and increased blood flow is referred to as neurovascular coupling. An increase in the BOLD signal is a result of increased oxygenated haemoglobin caused by neural activity and consequent changes in cerebral blood flow. There are multiple methodologies utilised in analysing resting state fMRI including seed-based, ICA-based and graph theory-based approaches (van den Heuvel and Hulshoff Pol, 2010). Given that neural oscillatory activity is thought to underlie the BOLD response, it is not surprising that similarities have been found between measures of connectivity using magnetoencephalography (MEG) and BOLD fMRI, both during task performance (Luckhoo et al., 2012) and at rest (Brookes et al., 2011). However, whilst fMRI gives excellent spatial resolution, it is an indirect measure of neural activity with poor temporal resolution. As a result, non-neural, physiological confounds such as movement, cardiac and respiratory activity can influence apparent associations (Murphy et al., 2013).

Results from ICA-based resting state fMRI studies of schizophrenia are inconsistent whereas the majority of the seed-based fMRI studies show reduced connectivity in schizophrenia (Yu et al., 2012). When including task based fMRI studies, the picture is one of reduced functional connectivity in schizophrenia, particularly in frontal regions (reviewed by (Pettersson-Yeo et al., 2011).

In contrast to fMRI, MEG is a direct measure of neural activity with excellent temporal resolution and can therefore be considered complementary to fMRI (Brookes et al., 2011). There are multiple methods for investigating resting-state connectivity in MEG, one of which is amplitude envelope correlation. This method of analysis has been found to be robust and repeatable (Colclough et al., 2016) and successful in identifying differences between patients with schizophrenia and healthy controls (Brookes et al., 2016).

There are a limited number of MEG resting-state studies of schizophrenia and again, methodologies are heterogeneous (Bowyer et al., 2015, Canive et al., 1998, Fehr et al., 2003, Hinkley et al., 2011, Kim et al., 2014, Rutter et al., 2009, Sperling et al., 1998). Only some of these studies have used connectivity metrics in their analysis (Bowyer et al., 2015, Hinkley et al., 2011, Kim et al., 2014), the others using spectral analysis. Results of MEG resting-state connectivity studies are mixed with one study finding hyper-connectivity (Bowyer et al., 2015), one finding hypo-connectivity (Kim et al., 2014) and another finding both hypo and hyper-connectivity in different brain regions (Hinkley et al., 2011) in schizophrenia.

3.3 Aims and Hypotheses

Given these inconsistent findings, I sought to further explore resting-state connectivity in two groups of individuals with schizophrenia. The majority of studies have found reduced connectivity in schizophrenia (Pettersson-Yeo et al., 2011) and therefore, I tested the

hypothesis that participants with schizophrenia would show reduced resting-state connectivity compared with matched healthy control participants.

In this chapter, data will be reported from two studies; one conducted at Cardiff University and the other conducted as part of the University of Nottingham's Multi-modal Imaging Study in Psychosis (MISP) (Robson et al., 2016). Rather than combine the data, analysis was carried out on both groups separately in order to look for consistency in results between the two studies. Amplitude-amplitude coupling in resting-state MEG was used in both groups to explore the dysconnectivity hypothesis of schizophrenia. For one group, I also used amplitude-amplitude coupling in resting-state fMRI to explore both the dysconnectivity hypothesis and the complementarity of fMRI and MEG. To my knowledge, only one other study has explored resting-state connectivity using both fMRI and MEG in the same participant group (Houck et al., 2017). In contrast to Houck et al. (2017), who used an ICA based approach, I used an AAL atlas based amplitude envelope correlation approach. In combining this with similar analysis of fMRI resting-state data I sought to gather complementary information regarding both electrophysiological network connectivity and haemodynamic connectivity in a patient population.

3.4 Materials and Methods

3.4.1 Participants

Both studies were ethically approved in line with local and national practices (REC reference: Study 1- 10/WSE03/48, Study 2- 12/WM/0307). Participants in both studies gave written, informed consent prior to taking part.

Since data were collected as part of two different studies in two different sites, study protocols around recruitment and behavioural measures were somewhat different and so I will outline both studies separately.

3.4.1.1 Study 1

The data used in this study were acquired as part of a multi-modal imaging study of schizophrenia previously conducted at Cardiff University. 28 participants with a DSM-IV diagnosis of schizophrenia (20 males, 8 females; mean age: 44.6 +/-8.3, age range: 22-58) took part in the study. An age and sex matched group of 30 healthy control participants (18 males, 12 females; mean age 41.8+/-10.6, age range: 25-58) were recruited locally through a University noticeboard.

Participants with schizophrenia were recruited from an existing database of individuals that had previously taken part in the Cardiff Cognition in Schizophrenia Study (Rees et al., 2014). As part of the previous study, they were formally diagnosed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (Wing et al., 1990) and DSM-IV criteria (APA, 1994). This diagnosis was then verified by clinical consensus.

Inclusion criteria were: age between 16 and 75; English as a first language; normal or corrected vision; ability to give informed consent and for the case group, a DSM IV diagnosis of schizophrenia. Exclusion criteria were: a diagnosis of epilepsy or any severe neurological event such as head injury with loss of consciousness or clinically identified complications; any metal in their body that would preclude MRI or interfere with MEG scanning; for healthy controls, history of affective or psychotic disorder in themselves (assessed via administration of the MINI) or a first degree relative.

The Schedule for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990) was administered to the case group, which then informed ratings on the Scale of the Assessment

of Positive Symptoms (SAPS) (Andreasen, 1984) and the Scale of the Assessment of Negative Symptoms (SANS) (Andreasen, 1983). These assessment tools are used to rate the positive and negative symptoms associated with schizophrenia.

3.4.1.2 Study 2

For this study, data were acquired as part of the University of Nottingham's Multi-modal Imaging Study in Psychosis (MISP) (Robson et al., 2016).

28 cases with a DSM-IV diagnosis of schizophrenia (21 males, 7 females; mean age: 26.7 +/- 5.8, age range 18-44) took part in the study. An age, sex and socio-economic background matched group of 29 healthy control participants (22 males, 7 females; mean age: 27.3 +/- 6.7, age range 18-48) also took part. Controls had no history of neurological illness.

A symptom severity score of persisting symptoms was derived for each case using the SSPI (Liddle et al., 2002), a variant of the digit symbol substitution test and the SOFAS as outlined in Palaniyappan et al. (2013). This was calculated by extracting the first principle component using PCA and allowed quantification of the three main syndromes of schizophrenia: reality distortion, psychomotor poverty and disorganisation. (The extraction of the symptom severity score was completed by the team in Nottingham.)

3.4.2 MRI Data Acquisition

Individual anatomical MRIs (1-mm isotropic, T1-weighted (Cardiff: FSPGR, Nottingham: MPRAGE) were acquired using a 3.0 T MRI scanner (Cardiff: General Electric, Nottingham: Philips).

3.4.3 Functional MRI Procedure and Data Acquisition for Study 1

fMRI data was available for 27 cases and 30 controls, due to this not being performed in the initial stages of the study.

An eyes-open, resting-state scan lasting 10 min was acquired using a BOLD-weighted gradient-echo echo-planar imaging sequence (TR/TE=3000/35ms; FOV= 20.5cm; 53 slices, slice thickness=3.2mm; resolution=3.2mm isotropic, 200 volume acquisitions).

Resting-state functional data were de-spiked (using 3dDespike), time-shifted to a common temporal origin (using 3dTshift), brain extracted (using 3dAutomask) and volume registered (using 3dvolreg – all programs taken from the AFNI software package, http://afni.nihm.nih.gov/afni (Cox, 1996)). The mean functional volume for each subject was registered to the corresponding high-resolution T1-weighted image and normalised to the MNI-152 brain template (MNI152, nonlinearly derived, McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada) using flirt from the FSL package. The functional data were transformed into MNI space. White matter (WM) and cerebral spinal fluid (CSF) masks were calculated from the anatomical data using 3dSeg and were eroded by 1 voxel. The functional data were cleaned by regressing in one step the following (using 3dTproject): motion parameters and their derivatives; GM and CSF time-series calculated by averaging over the eroded mask; a set of sines and cosines for bandpass filtering between 0.01Hz and 0.1Hz; and a set of regressors to censor time points with motion greater than a Euclidean norm of 0.2. This resulted in 30 controls and 23 cases with adequate quality data for further analysis. Low frequency fluctuations were then extracted from 90 regions of interest as defined by the AAL atlas (Tzourio-Mazoyer et al., 2002). These were then used in subsequent analysis described below.

3.4.4 MEG Procedure and Data Acquisition

Identical 275-channel axial gradiometer CTF systems (VSM MedTech) were used at both centres to collect MEG data. For Study 2 (MISP) participants were orientated supine. For Study 1, participants were seated upright in the scanner. Data were acquired at a sampling frequency of 1200Hz for study 1 and 600Hz for study 2. Electromagnetic coils were placed at three fiduciary locations (bilateral pre-auricular and nasion) and their position relative to the MEG sensors was localised before and after each session. For study 1, MEG data was co-registered to the individual anatomical MRI of each participant by marking the positions of the fiducial coils on each MRI. For study 2, a 3D digitiser (Polhemus Inc., Vermont) was used to obtain a three-dimensional digitation of the participants' head shape, relative to the fiducial markers.

For study 1, participants completed two resting state tasks, each lasting 5 minutes; one with their eyes open and focused on a central red fixation point and the other with their eyes closed. For study 2, participants completed one 10 minute, eyes open resting state task.

Datasets were down-sampled to 600Hz (where required); band-pass filtered at 1-150Hz and segmented into 2 second epochs. Each epoch was then visually inspected for artefacts such as large muscle contractions or movement and if present, excluded from subsequent analysis. Datasets were filtered into the following bandwidths: Delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), low gamma (30–50 Hz) and high gamma (50-90 Hz).

3.4.5 Pipeline for Amplitude-Amplitude Coupling

Figure 3.1 outlines the methods for data analysis used in the studies. Using the Automated Anatomical Labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002), 90 sources of interest in the cortex and subcortical regions were identified from which time-series were extracted and analysed. MEG data for each participant and frequency band were source reconstructed using a linearly constrained minimum variance (LCMV) beamformer on a 6mm grid. Single shell source models were registered to the standard co-ordinate space of the Montreal Neuroimaging Institute. Orthogonalisation of signals using linear regression was used to reduce any zero-time lag correlation suggestive of signal leakage thereby reducing any artefactual correlation (Colclough et al., 2015). The Hilbert envelope (amplitude envelope) of source-space neural oscillatory activity in each frequency band for every AAL region was then computed from orthogonalised signals. A median filter was applied to smooth out large deflections in the data. The Hilbert Envelope with the maximum percentage change within each AAL region was then used for further analysis.

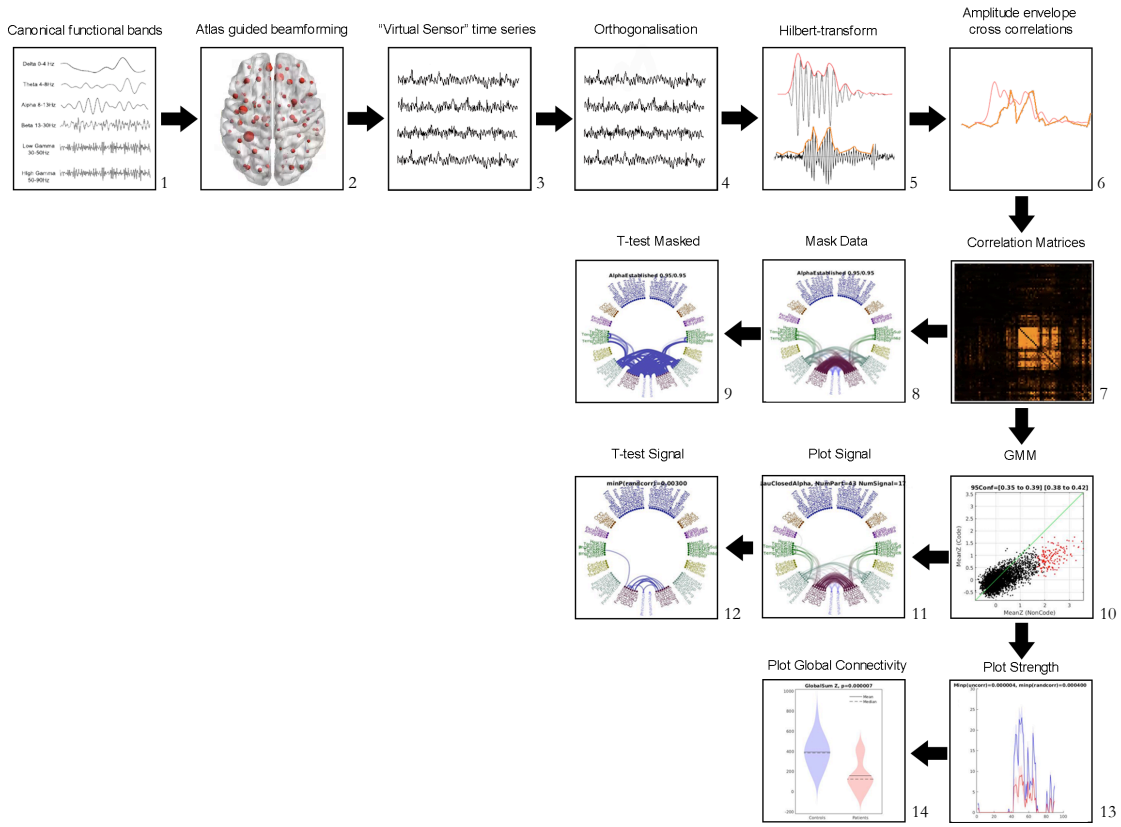


Figure 3.1 MEG analysis pipeline for resting-state functional connectivity.
1-7. We used an AAL atlas-guided beamforming approach to define 90 cortical and subcortical seed regions. We first filtered data into canonical functional bands and then reconstructed virtual sensor time series in each of the 90 AAL regions. The data was orthogonalised to suppress source leakage. We applied a Hilbert transform to give estimates of amplitude and phase for each of these regions. Amplitude envelopes were then cross-correlated to derive the degree of connectivity between AAL regions. z statistics were derived from the correlation coefficients and used to construct connectivity matrices.
8-9. Data was initially masked to show only the highest 5% of connections and this was followed by a t-test of these connections.
10-12. A Gaussian mixture modelling approach was also used to define connections as signal or noise. "Signal" connections were plotted onto connectivity maps, followed by t-tests of these connections. (Blue=lower connectivity in these connections in cases.)
13. Strength of connectivity was calculated by summing z statistics of signal connections horizontally. (Red=Cases. Blue=Controls)
14. Global connectivity was calculated by summing all z statistics of signal connections. Mean, median and t-test. (Red=Cases. Blue=Controls)

3.4.6 Statistical Analyses

Amplitude envelope cross-correlations for every pair of AAL regions were computed across the entire resting state run for each frequency band for MEG time series. This gave a single value (correlation coefficient) for each pair of AAL regions, quantifying the degree of

amplitude correlation between them. A variance stabilising transformation, (Fishers Z transformation) was applied to the correlation coefficients to normalise their behaviour and reduce estimation bias when averaging (Silver and Dunlap, 1987). The theoretical variance of the z statistic was then calculated in order to transform Fishers Z scores into z statistics. This transformation corrects for the different number of trials in different groups. Connectivity matrices were constructed using z statistics as edge weights for all AAL atlas regions. Resulting matrices are symmetrical, with each square representing the z statistic of connectivity between one brain region and another. Connectivity matrices were constructed for every participant for every frequency band. Averaged, uncorrected group-wise data was then plotted onto connectivity matrices to visually compare connectivity between groups.

In order to reduce the number of comparisons, those connections with a z statistic within the highest 5%, present 95% of the time in the bootstrapping procedure were considered the most prominent connections and plotted onto a circular map. This was followed by group comparisons of these strongest connections.

A further analysis method involved classifying connections into signal or noise using a statistical thresholding procedure based on Gaussian mixture modelling (GMM) as discussed in Chapter 2 (Plataniotis and Hatzinakos, 2000). Using the Expectation-Maximisation algorithm with two components randomly selected as the initial component means, a Gaussian mixture distribution was fitted to the z statistics by maximum likelihood. In each frequency band, only connections with a greater than 50% probability of being within the signal were accepted as valid.

The signal z statistic for every region was summed horizontally to give the strength of connectivity of each region. Two-tailed t-tests were used to evaluate between group

differences in strength and statistical significance was defined as a threshold of 0.05. Additionally, global connectivity was calculated and compared between groups by summing all signal connections.

Permutation testing of the maximum t-statistic was used to test for statistical differences between case and control groups. Case and control labels were swapped randomly, creating two artificial matrices, which were then compared. This was repeated 2000 times to generate a null distribution. This was then compared with the value from the real result to ascertain the probability that the result occurred by chance. This is called omnibus testing and automatically controls for multiple comparisons.

These same analysis procedures were also used for the fMRI BOLD time-series in Study 1. After carrying out the GMM procedure on both the MEG and fMRI data, connections were found that were valid for both. Those connections were then analysed further and cases compared with controls. The uncorrected t-statistic for fMRI and MEG were then plotted against each other to explore associations. Since the fMRI data was collected in the eyes-open condition, I compared this only to the eyes-open MEG data.

3.5 Results

3.5.1 Demographic and Clinical Information

Table 3.1 shows participant demographic and clinical information, including medication, reported as mean daily dose equivalents (DDD) (WHO, 2012), mean SAPS and SANS scores for study 1 and disease severity scores for study 2. DDD refers to the average daily maintenance dose for a drug. DDD for antipsychotics was calculated by dividing the participants prescribed daily dose by the average daily maintenance dose of that drug. For

those on multiple antipsychotic medications, these were added together. Mean DDD across cases was then calculated by averaging these values across the cohort (Sweileh et al., 2014).

For study 1, all except for one case were treated with antipsychotic medication. Both SAPS and SANS scores overall were low suggesting a group of cases with minimal symptoms. For study 2, all cases were treated with antipsychotic medication. Participants in study 1 were significantly older than those in study 2 ($p<0.001$)

	Study 1		**Study 2**	
	Schizophrenia n=28	Control n=30	Schizophrenia n=28	Control n=29
Age (Mean (SD))	44.67(8.31)	41.86(10.66)	26.7(5.8)	27.3(6.7)
Gender (M(F))	20(8)	18(12)	21(7)	22(7)
Antipsychotic DDD (Mean (SD))	1.17(0.82)	---	1.24(0.69)	---
SAPS (Mean (SD))	3.46(3.89)	---	---	---
SANS (Mean (SD))	3.96(2.77)	---	---	---
Illness Severity	---	---	0.25(0.88)	---

Table 3.1 Demographic and clinical information
S.D = standard deviation, M=male, F= female, DDD= mean daily dose equivalents, SAPS= Scale of the Assessment of Positive Symptoms, SANS= Scale of the Assessment of Negative Symptoms

3.5.2 Study 1: Resting State Functional Connectivity Analysis: Uncorrected Analysis of Differences

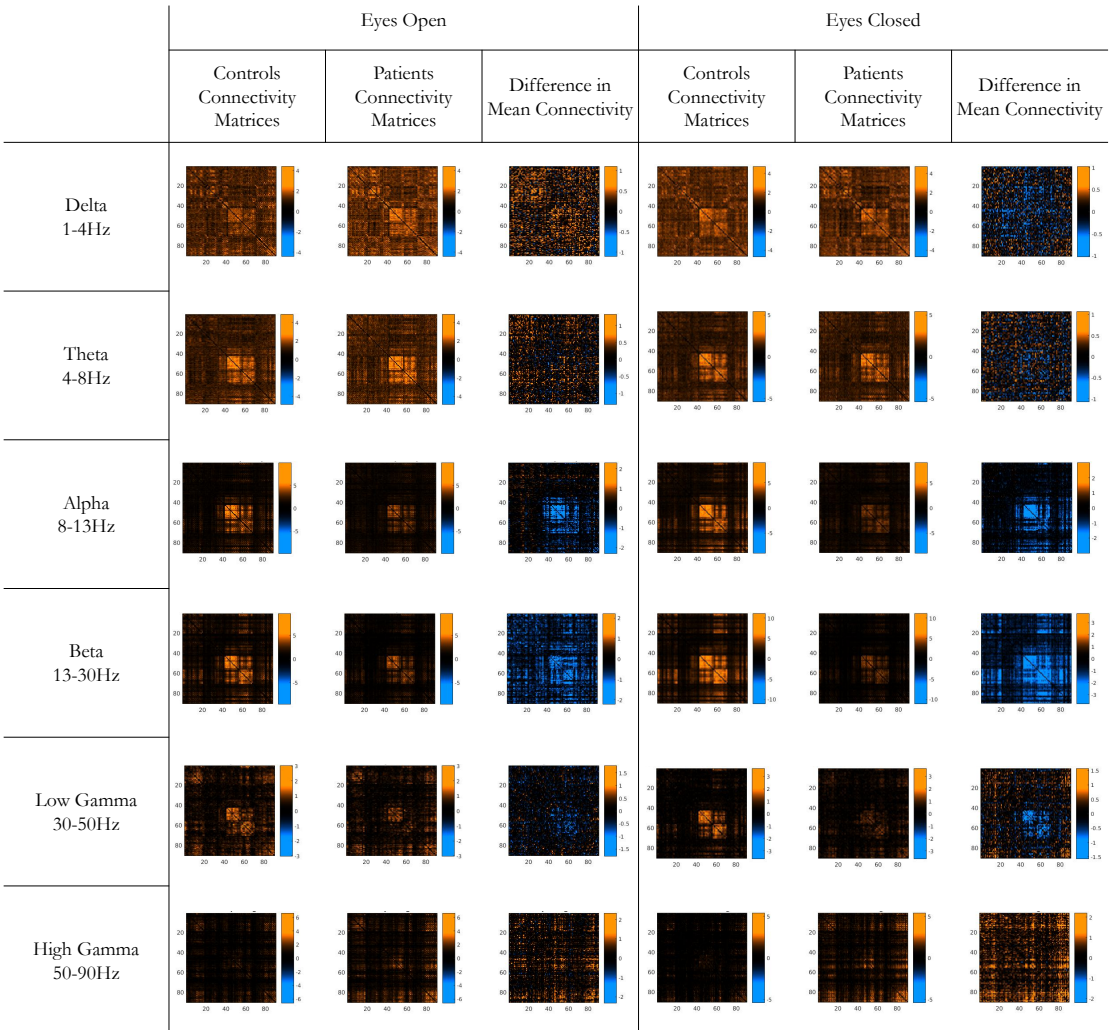


Figure 3.2: Study 1 Connectivity matrices.
Left panel = Eyes Open, Right panel = Eyes Closed. Orange=higher connectivity. Blue=lower connectivity. x axes=AAL nodes 1-90, y axes=AAL nodes 1-90

Figure 3.2 shows grouped connectivity matrices for cases and controls and difference in mean connectivity for study 1 for all frequency bands. The left panel shows results for the eyes-open condition and the right panel shows results for the eyes-closed condition.

For most frequency bands, particularly theta, alpha, beta and low gamma, there is a hub region of increased connectivity (represented by a central block of orange on the connectivity matrices) that represents connectivity in the posterior parietal and occipital regions. This is evident for both cases and controls. Uncorrected comparisons between cases and controls shows reduced connectivity in cases in the alpha and beta frequency bands. This is represented by blue regions and appears to be particularly prominent in the posterior parietal hub region previously discussed. Differences in other frequency bands appear much noisier with more widespread differences.

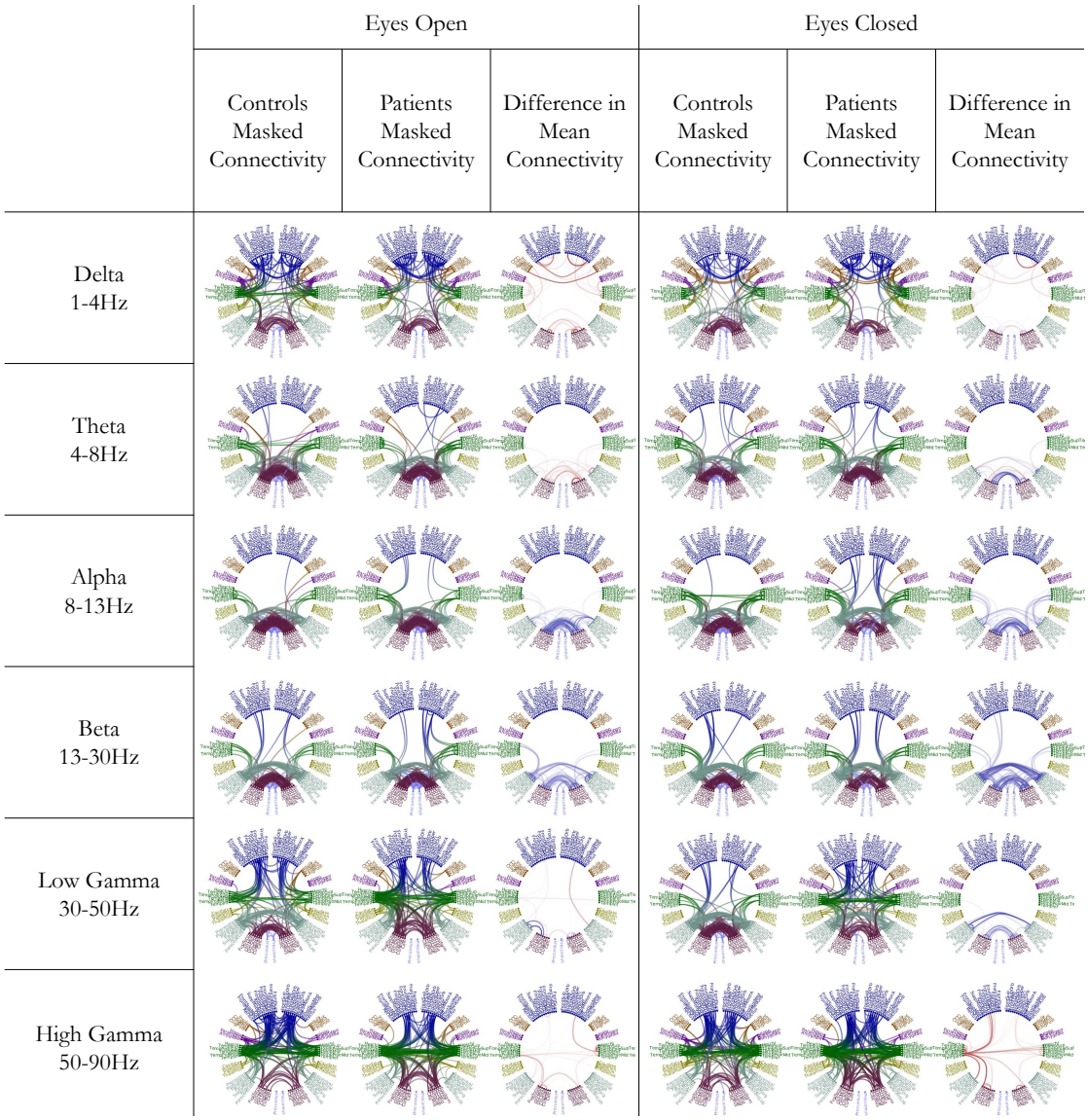


Figure 3.3: Uncorrected connectivity maps.
Left panel = Eyes Open. Right panel = Eyes Closed.
Masked connectivity refers to the connections with the highest 5% z-statistics, present 95% of the time in the bootstrapping procedure.
Controls Masked Connectivity = mean highest connections over all controls.
Patients Masked Connectivity = mean highest connections over all patients.
Difference in Mean Connectivity= Patients Masked Connectivity minus Controls Masked Connectivity.
Blue=lower connectivity in cases. Red = higher connectivity in cases.

Figure 3.3 shows circular connectivity maps for study 1 using the data masking procedure outlined above. Each point on the connectivity map represents an AAL node. Similar patterns of connectivity are evident in both cases and controls with again, posterior parietal and occipital connections being the most consistent connections as well as fronto-temporal

and parieto-temporal connections. When calculating the difference in mean connectivity, there is a consistent reduction in connectivity in these occipital and parietal connections in the alpha and beta frequency band.

3.5.3 Study 1: GMM Analysis

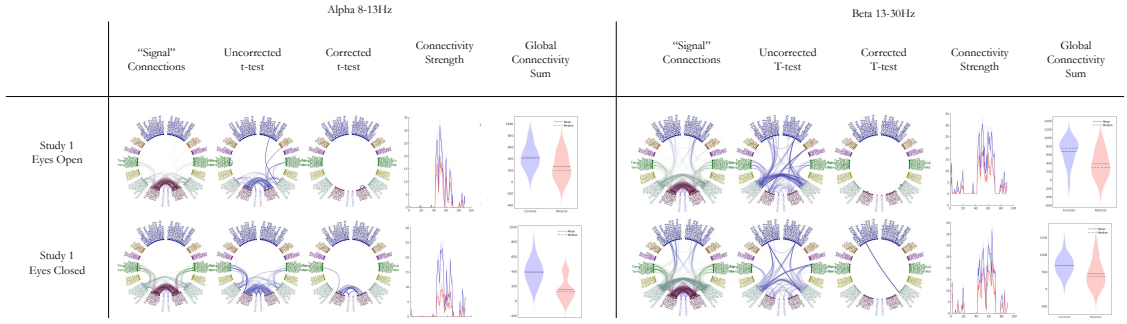


Figure 3.4: Study 1 GMM Analysis.
Left panel = Alpha (8-13Hz). Right panel = Beta (13-30Hz)
Signal connections= connections found to be in the "signal" Gaussian using GMM.
Uncorrected t-test= uncorrected t-test of signal connections between cases and controls. Red lines=increased connectivity. Blue lines= decreased connectivity in cases.
Corrected t-test= corrected t-test of signal connections between cases and controls. Red lines=increased connectivity. Blue lines= decreased connectivity in cases.
Connectivity Strength= Sum of every signal connection for each AAL node. Horizontal axes=AAL node. Vertical axes=Connectivity strength. Red=Cases. Blue=Controls.
Global Connectivity Sum= Violin plot of sum of all signal connections for cases and controls. Red=Cases. Blue=Controls.

GMM analysis did not reveal a consistent pattern of "signal" and "noise" nodes for the delta, theta, low gamma or high gamma frequency bands. In these frequency bands, there were no node-node connections identified that had a mean probability of being in "signal", averaged across participants, of greater than 50%. Therefore, data will only be presented for the alpha and beta frequency bands and are shown in Figure 3.4. Using this approach, "signal" connections were found predominantly in occipital, parietal and temporal regions in the alpha frequency band. In the beta frequency band, signal connections were more widespread and in addition included fronto-parietal and temporo-parietal connections. In both the eyes-open and eyes-closed conditions, posterior (occipital and parietal) connections were

consistently reduced in cases in the alpha frequency band. Randomisation testing of the t-statistic identified the following connections as being significantly ($p<0.05$, corrected) reduced in cases:

Alpha Eyes-Open (one connection):

Right Superior Occipital (50) to Right Fusiform (56)

Alpha Eyes-Closed:

Multiple occipito-occipital connections plus left fusiform to left mid temporal
Right Calcarine (44) to Left Superior Occipital (49)
Left Cuneus (45) to Right Cuneus (46)
Left Cuneus (45) to Left Superior Occipital (49)
Left Cuneus (45) to Right Superior Occipital (50)
Left Cuneus (45) to Right Mid Occipital (52)
Left Cuneus (45) to Left Inferior Occipital (53)
Left Cuneus (45) to Left Precuneus (67)
Right Cuneus (46) to Left Superior Occipital (49)
Right Cuneus (46) to Left Mid Occipital (51)
Right Cuneus (46) to Left Precuneus (67)
Left Lingual (47) to Left Mid Occipital (51)
Left Lingual (47) to Left Inferior Occipital (53)
Right Lingual (48) to Left Inferior Occipital (53)
Left Superior Occipital (49) to Left Mid Occipital (51)
Left Superior Occipital (49) to Left Inferior Occipital (53)
Left Superior Occipital (49) to Left Precuneus (67)
Right Superior Occipital (50) to Right Mid Occipital (52)
Left Fusiform (55) to Left Mid Temporal (85)

In the beta frequency band, uncorrected reduced connectivity in cases was more widespread and included multiple fronto-parietal and homologous frontal connections. The following connections were significantly reduced in cases after correction for multiple comparisons:

Beta Eyes-Open:

Left Superior Occipital (49) to Left Mid Occipital (51)
Right Inferior Parietal (62) to Right SupraMarginal (64)

Beta Eyes-Closed:

Left Supplementary Motor Area (19) to Right Paracentral Lobule (70)

Connectivity strength was significantly reduced in cases in both conditions.

Alpha Eyes-Open (p=0.0148)

Right Superior Occipital (50)
Left Mid Occipital (51)

Alpha Eyes-Closed (p<0.001)

Left Cuneus (45)
Right Cuneus (46)
Left Lingual (47)
Left Superior Occipital (49)
Right Superior Occipital (50)
Left Mid Occipital (51)
Right Mid Occipital (52)
Left Inferior Occipital (53)
Right Inferior Occipital (54)
Left Angular Gyrus (65)

Beta Eyes-Open (0.005)

Left Mid Occipital (51)
Left Superior Parietal (59)
Right Inferior Parietal (62)
Left SupraMarginal (63)
Left Angular Gyrus (65)

Beta Eyes-Closed (0.02)

Left Angular Gyrus (65)

Global connectivity strength was also reduced in both conditions and frequency bands (Alpha: eyes open, $p=0.006$, eyes closed, $p<0.001$. Beta: eyes open, $p<0.001$, eyes closed, $p=0.009$).

3.5.4 Study 1: fMRI Results

fMRI results can be seen in figure 3.5. Uncorrected differences between cases and controls show predominantly increased connectivity in cases occipitally and parietally. However, these differences did not reach statistical significance.

3.5.5 Study 1: Comparison between MEG and fMRI Results

Since valid connections were only found in the alpha and beta frequency bands using the GMM procedure in study 1, only these frequency bands were compared with the fMRI data.

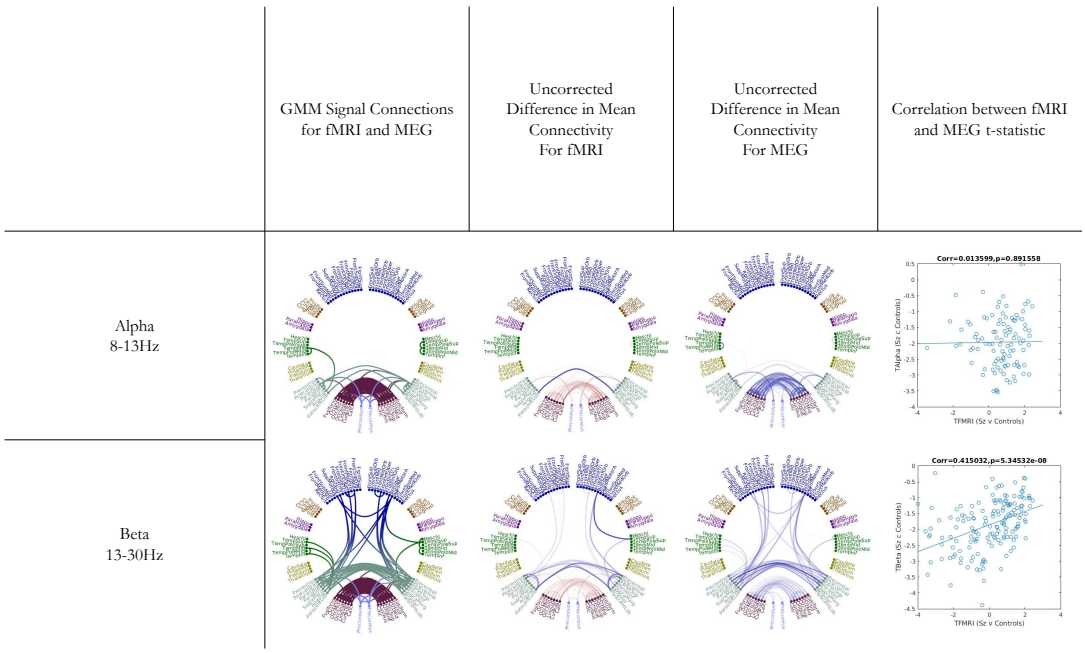


Figure 3.5: fMRI vs MEG connectivity
Top panel= alpha, lower panel=beta
GMM Signal connections= Connections identified as "signal" in GMM procedure for both fMRI and MEG.
Difference in Mean Connectivity= Patients Connectivity minus Controls Connectivity. Blue=lower connectivity in cases. Red = higher connectivity in cases.
Correlation between fMRI and MEG t-statistic.

Figure 3.5 shows comparisons between fMRI and MEG in the alpha and beta frequency bands. In the alpha frequency band, signal connections present in both fMRI and MEG data were predominantly in occipital and parietal regions. Overall, the difference in these connections for fMRI revealed increased connectivity in cases, whereas, as discussed previously, reduced connectivity was found in MEG. However, for fMRI, these differences did not reach statistical significance. When looking at correlations across node-node connections, between t-tests in MEG and fMRI, there was no correlation in the alpha frequency band.

In the beta frequency band, signal connections present in both fMRI and MEG data were predominantly in occipital and parietal regions as well as fronto-parietal and temporo-parietal. Increases and decreases in connectivity in cases in these connections were evident in the fMRI data. Again, these differences were non-significant. MEG data revealed reduced connectivity in these connections in cases. There was a significant correlation between t-tests of MEG beta connectivity between cases and controls and fMRI connectivity. i.e. those connections showing the biggest reductions in connectivity in cases in fMRI also show the biggest reductions in connectivity in the MEG beta frequency band.

3.5.6 Study 2: Resting State Functional Connectivity Analysis: Uncorrected Analysis of Differences

Given that robust connections were only found in the alpha and beta frequency bands in study 1, only these frequency bands were explored in study 2.

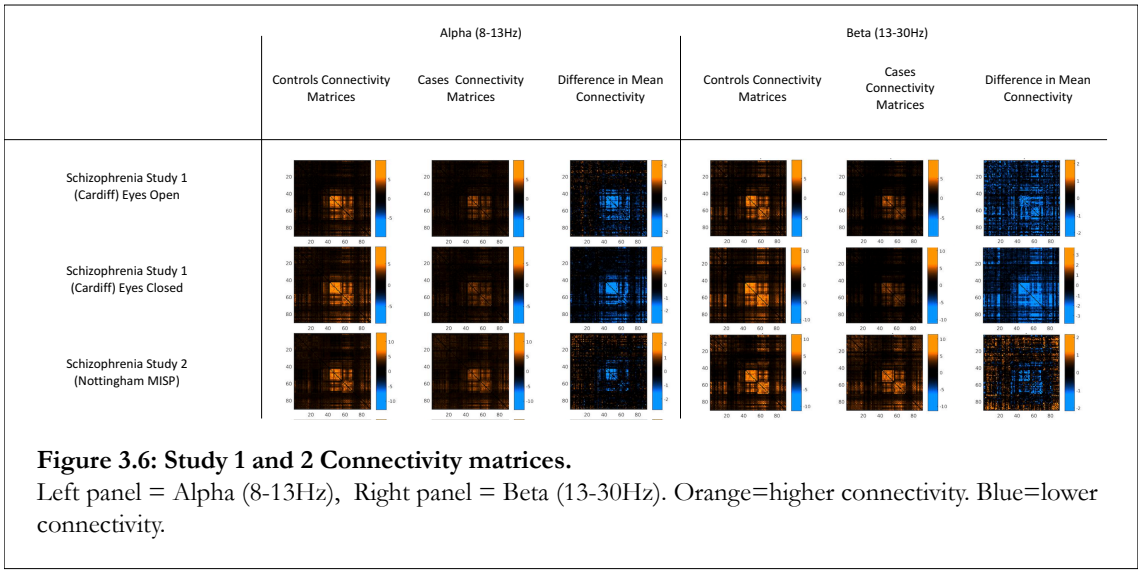


Figure 3.6: Study 1 and 2 Connectivity matrices.
Left panel = Alpha (8-13Hz), Right panel = Beta (13-30Hz). Orange=higher connectivity. Blue=lower connectivity.

Figure 3.6 shows grouped connectivity matrices for cases and controls and difference in mean connectivity for study 1 and 2 for alpha and beta frequency bands. The left panel shows

results for the alpha frequency band and the right panel shows results for the beta frequency band. Again, a hub region of increased connectivity (orange central area corresponding to posterior parietal region) is seen in study 2 in both the alpha and beta frequency bands in both cases and controls. (When comparing with results for study 1, patterns of connectivity appear very similar.)

In study 2, uncorrected comparisons between cases and controls shows reduced connectivity in cases in the alpha and beta frequency bands, again in the posterior parietal hub region previously discussed. However, there also appear to be regions of more widespread increased connectivity in study 2, particularly in the beta frequency band.

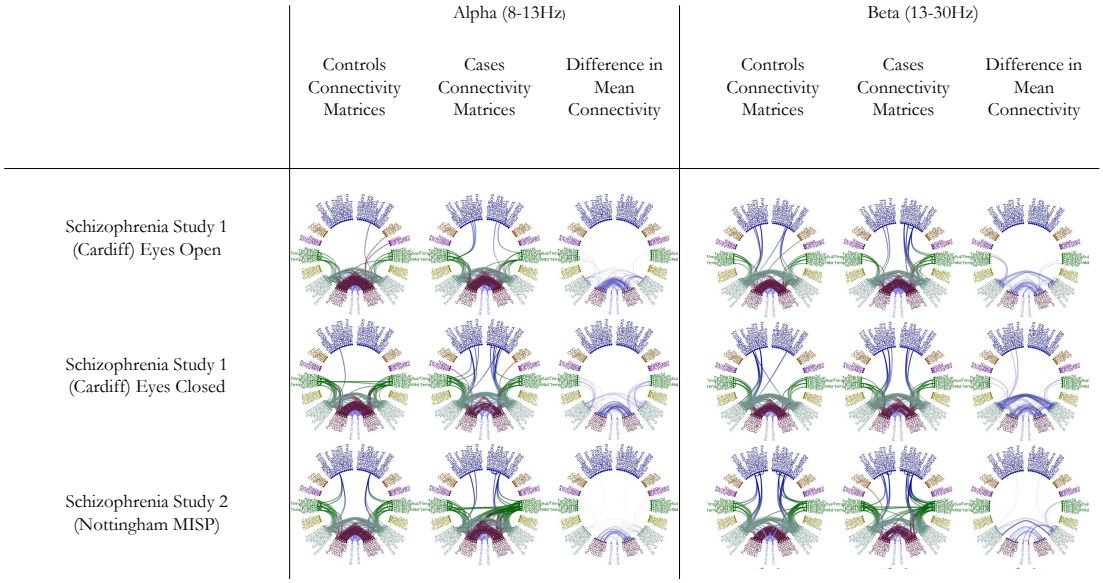


Figure 3.7: Study 1 and 2 Uncorrected connectivity maps
Left panel = Alpha (8-13Hz). Right panel = Beta (13-30Hz)
Masked connectivity refers to the connections with the highest 5% z-statistics, present 95% of the time in the bootstrapping procedure.
Controls Masked Connectivity = mean highest connections over all controls.
Cases Masked Connectivity = mean highest connections over all patients.
Difference in Mean Connectivity= Cases Masked Connectivity minus Controls Masked Connectivity.
Blue=lower connectivity in cases. Red = higher connectivity in cases.

Figure 3.7 shows circular connectivity maps for studies 1and 2 in the alpha and beta frequency bands using the data masking procedure outlined above. The left panel shows the alpha frequency band and the right shows the beta frequency band. Again, similar patterns of connectivity are seen in cases and controls in study 2 as those seen in study 1. Uncorrected differences in mean connectivity show similar patterns of reduced connectivity in cases in the alpha and beta frequency bands in study 2 to those seen in study 1.

3.5.7 Study 2: GMM Analysis

Results between study 1 and 2 diverge somewhat when analysing the data using a GMM approach.

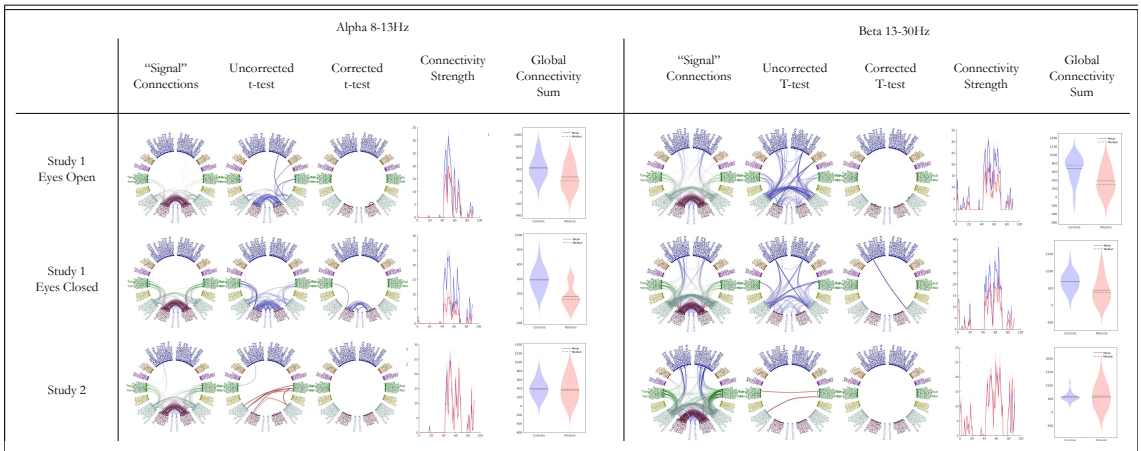


Figure 3.8: Study 1 and 2 GMM Analysis
Left panel =Alpha (8-13Hz), Right panel=Beta (13-30Hz)
Signal connections= connections found to be in the "signal" Gaussian using GMM.
Uncorrected t-test=uncorrected t-test of signal connections between cases and controls. Red lines=increased connectivity. Blue lines=decreased connectivity in cases.
Connectivity Strength=Sum of every signal connection for each AAL node. Horizontal axes=AAL node. Vertical axes=Connectivity strength. Red=cases. Blue=controls.
Global Connectivity Sum= Violin plot of sum of all signal connections for cases and controls. Red=cases. Blue=controls.

GMM analysis results in the identification of similar "signal" connections in study 2 to those found in study 1. An uncorrected t-test comparing these connections in cases and controls results in increased temporo-parietal and parieto-occipital connectivity in cases in the alpha frequency band. In the beta frequency band, it results in two connections with increased connectivity in cases. However, after correcting for multiple comparisons, there were no significant differences between cases and controls in the alpha and beta frequency bands for study 2. This was also the case when exploring connectivity strength and global connectivity.

3.6 Correlations with clinical scores

3.6.1 Study 1

3.6.1.1 Eyes Open

In the alpha frequency band, there was one occipital connection that correlated with SAPS, however, this did not reach statistical significance after permutation testing to correct for multiple comparisons across connections. There were no other correlations found between connectivity in the alpha or beta frequency bands and SANS, DDD or age.

3.6.1.2 Eyes Closed

In the beta frequency band, there was one parietal connection that negatively correlated with SAPS, however, this did not reach statistical significance. There was also one parietal connection that negatively correlated with age in this frequency band but again, this did not reach statistical significance. Aside from these two correlations, there were no correlations found between connectivity and SAPS, SANS, DDD or age.

3.6.2 Study 2

In the alpha frequency band, connectivity in multiple parietal-occipital connections and one temporo-parietal connection negatively correlated with illness severity. However, these did not reach statistical significance. Results were similar for the beta frequency band with fairly widespread connectivity being negatively correlated with illness severity. After randomisation testing, connectivity in one connection between the right angular gyrus and left mid temporal negatively correlated with illness severity (Figure 3.9).

In the beta frequency band DDD negatively correlated with connectivity in two occipital connections. However, after randomisation testing, these did not meet statistical significance.

In the alpha frequency band, there were no correlations between connectivity and age. In the beta frequency band, age positively correlated with connectivity in multiple temporo-parietal and temporo-occipital connections. However, after randomisation, these did not meet statistical significance.

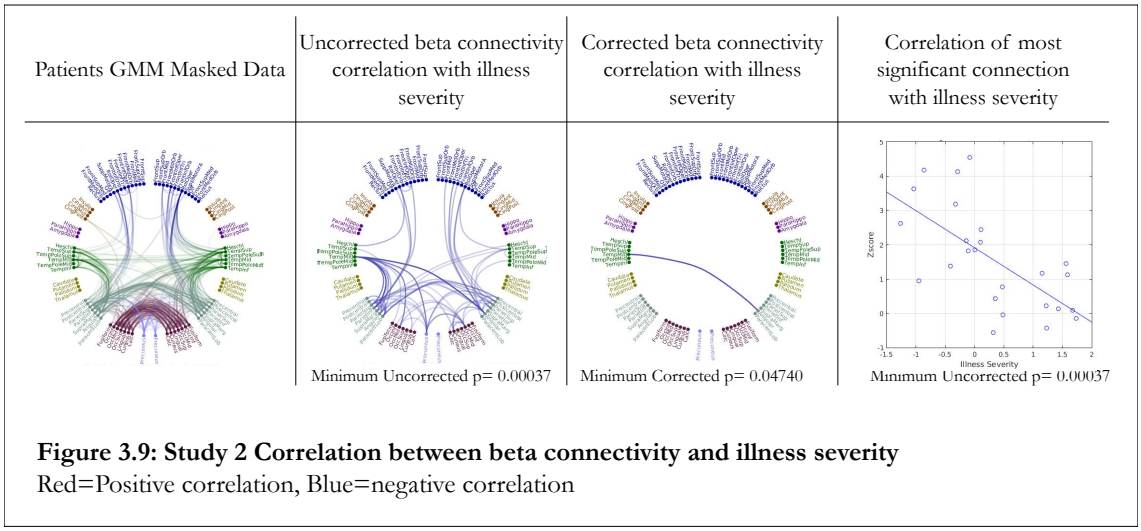


Figure 3.9: Study 2 Correlation between beta connectivity and illness severity
Red=Positive correlation, Blue=negative correlation

3.7 Discussion

Resting-state dysconnectivity has repeatedly been found in patients with schizophrenia. However, few studies have used MEG to probe resting-state connectivity in schizophrenia and of those that have, results are heterogeneous (Bowyer et al., 2015, Hinkley et al., 2011, Kim et al., 2014). In this study, amplitude-amplitude coupling, a robust and repeatable metric, was used to elucidate the link between schizophrenia and connectivity. In addition, a novel method of limiting analysis to only "signal" connections was used in order to reduce bias and threshold data.

Through this method, robust "signal" connections in the alpha and beta frequency bands were identified. Using this GMM approach, statistically significantly reduced connectivity was evident in one schizophrenia study (in two conditions) in the alpha and beta frequency bands. In a second study, we found no statistically significant differences between cases and controls when using this approach. Results from Study 1 were consistent with research finding dysconnectivity in schizophrenia, particularly those finding reduced parietal and occipital connectivity in patients with schizophrenia (Henseler et al., 2010, Zhuo et al., 2014, Wende et al., 2015). In addition, the finding of reduced connectivity specifically in the alpha and beta frequency bands is of interest. Using similar methodology, Brookes et al, 2016 found reduced alpha connectivity in patients with schizophrenia from the same cohort used in study 2 (MISP) when undertaking a motor task. Zumer et al. (2014) found that alpha oscillations are involved in gating information flow from visual areas to higher cortical centres. It may be, therefore that reduced alpha connectivity may represent a deficit in gating information flow in patients with schizophrenia that results in abnormal salience. This supports early theories such as that of Venables (1964) that the lack of control over the flood of sensory information (or input dysfunction) may be an underlying pathophysiological mechanism of schizophrenia (Freedman et al., 2002).

3.7.1 Inconsistencies between Study 1 and Study 2

There may be several explanations as to why there was a difference in results between the two studies. Firstly, the studies were developed and executed independently in two different sites. Therefore, the two cohorts may differ due to methodological differences during recruitment and data collection. This is evident when exploring the demographic/background of participants included in the two studies. There was a significant difference in age of participants between the two studies. Also, whilst not formally investigated (as data was not available for both studies), illness duration was heterogeneous in Study 2 (range: 7 months-12 years, mean: 4.6 years), whereas all cases recruited in Study 1 were recruited from a database of individuals with schizophrenia that had taken part in research (and therefore been diagnosed) many years earlier. It may be the case that age (and consequently illness duration) may account for differences in connectivity between studies. This is supported by a recent study by Sheffield et al. (2016) that found an age-related accelerated decline in functional connectivity in schizophrenia. In addition, Anticevic et al. (2015a), found hyper-connectivity in early schizophrenia and in healthy controls after administration of ketamine but hypo-connectivity in a group with chronic schizophrenia, suggesting that underlying neurobiology and consequently, connectivity may change through the course of the illness and therefore be affected by age. This is also supported by studies finding changes in structural connectivity (Friedman et al., 2008) and neurochemistry (Marsman et al., 2013) through the course of schizophrenia. However, there was no statistically significant correlation between age and connectivity in either study presented here and it is therefore difficult to firmly conclude that age and illness duration led to differences in results between studies.

There was a significant negative correlation in Study 2 between beta connectivity and illness severity. In this instance, illness severity is a holistic measure of the severity of the disorder and is calculated using current psychopathology, a measure of cognition and a measure of social and occupational functioning. It has been suggested that beta synchrony has a role in higher level cognition (Donner and Siegel, 2011) and this may therefore explain why illness severity negatively correlated with beta connectivity in study 2. Differences in illness severity may therefore also explain why there were significant differences in connectivity in cases in Study 1 but not in Study 2 (although this was not formally investigated as illness severity scores were not available for Study 1).

3.7.2 MEG vs fMRI

There were clear differences in results when comparing fMRI and MEG in study 1. Using MEG, there was reduced connectivity in cases, whereas, there were no statistically significant differences between cases and controls when using fMRI. Such differences in results between imaging techniques were also evident in a recent fMRI and MEG resting-state study of schizophrenia using ICA whereby the authors found frontal and temporal hyperconnectivity and hypoconnectivity in the PCC using MEG and hypoconnectivity using fMRI (Houck et al., 2017). In the current study however, when looking at correlations between t-tests of connectivity using the two modalities, I found a significant correlation between fMRI and beta frequency band MEG. This suggests that data collected using the two methodologies are complementary and that there is a link between haemodynamic network connectivity and electrophysiological network connectivity in schizophrenia. This is consistent with other work linking beta band activity and connectivity seen in MEG to fMRI (Garcés et al., 2016, Singh et al., 2002, Brookes et al., 2011).

3.7.3 Strengths

Whilst resting-state functional connectivity using both fMRI and MEG in schizophrenia has been previously explored (Houck et al., 2017), this is the first study to use AAL atlas-based amplitude envelope/BOLD time-series correlations to compare the two methods.

Using the same analysis pipeline for both methodologies and both studies conducted at both sites, has resulted in finding a consistent hub region of increased connectivity in both cases and controls.

Differences between cases and controls have been rigorously corrected for during the analysis stage, for example, the number of trials included after artefact rejection. In addition, the novel GMM based approach of thresholding data results in reduced bias due to differences in SNR between groups.

3.7.4 Limitations

There are several limitations to the study, which may account for differences seen between participants with schizophrenia and healthy controls. Firstly, antipsychotic medication exposure has been found to cause modulations in functional connectivity. (Kraguljac et al., 2016c, Bai et al., 2016, Lui et al., 2010). All participants with schizophrenia (except one case in Study 1) were prescribed antipsychotic medication and therefore, this could be a potential confounding factor. Therefore, differential functional connectivity may be due to differential antipsychotic exposure. However, in both studies presented here, there was no correlation between connectivity and DDD, suggesting that this may not be the case.

Antipsychotic exposure may also explain the different results found in fMRI and MEG. D2 blockade (an action of most antipsychotics) reduces dopamine-induced vasoconstriction (Krimer et al., 1998) and therefore, could have a differential effect upon haemodynamic networks as measured by BOLD.

Nicotine exposure may also have an impact upon connectivity (Wang et al., 2017, Hobkirk et al., 2017). Participants were not asked to refrain from smoking, therefore, acute effects of nicotine exposure cannot be ruled out. This should be taken into consideration in future studies of this nature.

3.8 Conclusions

In conclusion, reduced functional connectivity was found in one cohort of cases with schizophrenia in the alpha and beta frequency bands. This result was not replicated in a second cohort. However, in the second study, I found a significant negative correlation between beta band connectivity and illness severity suggesting connectivity in this frequency band is lower in more unwell patients. Therefore, this study supports the dysconnectivity hypothesis of schizophrenia and a link between connectivity and psychopathology. Furthermore, these results suggest that dysconnectivity in schizophrenia is frequency specific. In addition, whilst there was no correlation between age and connectivity in these studies, there was a significant difference in age (and possibly duration of illness) between our two groups which may account for differences in results between the two studies. Future studies could therefore explore this in further detail and in Chapter 5, I will do this by exploring functional connectivity in two groups of patients with differing duration of illness.

Whilst there were significant differences in cases with schizophrenia using MEG, there were no significant differences using fMRI. This may be due to the BOLD signal being a non-

specific measure that relates to all neural signals, only some of which will carry disease relevant information. The BOLD signal consists of synchronised activity at multiple frequencies (Magri et al., 2012, Scheeringa et al., 2011) and therefore may explain differential effects in fMRI and MEG. In addition, multiple factors affect the BOLD signal that are non-neural in origin such as anxiety, breathing rate, cardiovascular fitness, nicotine exposure, and eye movement artefacts all of which could differentially affect patients.

However, there was a positive correlation between t-tests of fMRI connectivity between cases and controls and beta band connectivity in MEG between cases and controls suggesting a link between the two measures.

Chapter 4 MEG Resting State Connectivity Modulations in Schizotypy in a Healthy Population

4.1 Rationale

The dimensional view of schizophrenia considers the disorder as existing on a continuum with subclinical symptoms of psychosis at one end (schizotypy) and clinically diagnosable schizophrenia at the other. This dimensional view is supported by genetic and neurobiological research (Reviewed by (Ettinger et al., 2014)). However, whilst there is substantial evidence supporting dysconnectivity in schizophrenia, there is very little research exploring resting-state connectivity in schizophrenia spectrum disorders (Wang et al., 2015, Zhang et al., 2015, Lagioia et al., 2010, Zhu et al., 2017). In this study, I sought to explore the continuum hypothesis of schizophrenia through looking at MEG resting-state connectivity in healthy participants with high and low schizotypy.

4.2 Background

Schizophrenia is a neuropsychiatric condition consisting of three core characteristic syndromes; reality distortion (positive symptoms), psychomotor poverty (negative symptoms) and disorganisation (cognitive). The diagnosis of schizophrenia is categorical, meaning that patients must meet certain criteria to receive a diagnosis (APA, 1994, WHO, 1993). However, the dimensional view of schizophrenia considers the disorder as existing on a continuum (Claridge and Beech, 1995) with sub-clinical psychotic traits in healthy individuals (schizotypy) (Rado, 1953) existing at one end of the spectrum and schizophrenia existing at the other. This dimensional view is supported by studies using factor analysis that

find three main dimensions of schizotypy that reflect those of schizophrenia (positive, negative and disorganised) (Bentall et al., 1989).

In addition, this continuum model is supported by other research which suggests that not only do schizotypy and schizophrenia share common psychopathology, there is also a genetic and neurobiological overlap (Reviewed by (Ettinger et al., 2014)). (However, as discussed in Chapter 1, findings of shared genetic predisposition in schizophrenia and schizotypy are limited with more evidence against this, particularly for positive symptoms.)

As discussed previously, it has been hypothesized that schizophrenia is a syndrome of dysconnectivity; referring to abnormal integration within and between brain regions and a significant body of research has sought to explore this (Friston and Frith, 1995, Pettersson-Yeo et al., 2011, Stephan et al., 2006, Stephan et al., 2009a). However, despite support for the continuum hypothesis of schizophrenia, very little research has explored functional connectivity across the schizophrenia continuum (Wang et al., 2015, Zhang et al., 2015, Lagioia et al., 2010). The exploration of functional connectivity in schizotypy is useful as it allows us to understand more about schizophrenia spectrum disorder without confounding factors such as medication.

As with schizophrenia, much of the resting-state functional connectivity research in schizophrenia continuum disorders has utilised fMRI. The most recent study by Zhu et al. (2017) found reduced functional connectivity between bilateral precuneus and contralateral parahippocampal gyrus in schizotypal personality disorder and a negative correlation between functional connectivity and total SPQ score. Other studies have found mixed patterns of dysconnectivity in schizophrenia continuum disorders with region dependent increases and decreases in connectivity (Wang et al., 2015, Zhang et al., 2014b, Lagioia et al.,

2010). To date, to my knowledge, there are no published resting-state MEG studies of schizotypy.

4.3 Aims and Hypotheses

Given the accumulating evidence for dysconnectivity in schizophrenia, supported by findings in Chapter 3, I sought to use similar methods to explore the biological validity of the continuum model of schizophrenia.

Whilst there are no consistent findings regarding functional connectivity in schizotypy (Wang et al., 2015, Zhang et al., 2014b, Lagioia et al., 2010, Zhu et al., 2017), I tested the hypothesis that there would be dysconnectivity in schizotypy reflecting shared biological factors with schizophrenia. Given that it is suggested that schizotypy may represent a less severe form of schizophrenia, I hypothesised that there would be an intermediate pattern of dysconnectivity in individuals with schizotypy as compared to previous findings in schizophrenia.

In this chapter, I will report data from two studies; one conducted at Cardiff University (UK MEG Partnership *(MR/K005464/1)* and 100 brains) and the other conducted at the University of Nottingham (UK MEG Partnership). I will use our previously validated method of amplitude-amplitude coupling in resting-state MEG to explore dysconnectivity in schizotypy and thus the biological validity of the continuum model of schizophrenia.

4.4 Materials and Methods

4.4.1 Participants

Both studies were ethically approved in line with local and (if required) national practices. Participants in both studies gave written, informed consent prior to taking part.

For both studies, participants were healthy individuals who were homogenous in age, ethnicity, education and handedness. Participants had no self-reported history of psychiatric or neurological conditions and reported no use of psychoactive drugs. For Study 1, 183 participants were recruited as part of the UK MEG Partnership project and 100 Brains study (Brealy et al., 2015) at Cardiff University. For Study 2, 70 participants were recruited as part of the UK MEG Partnership project at the University of Nottingham. Neither of these resting-state datasets had been previously analysed.

Participants were assessed using the Schizotypal Personality Questionnaire (SPQ) (Raine, 1991). This is a self-rated questionnaire consisting of 74 items that fall under nine subscales; ideas of reference, social anxiety, odd beliefs/magical thinking, unusual perceptual experiences, eccentric/odd behaviour and appearance, no close friends, odd speech, constricted affect, suspiciousness/paranoid ideation.

Group analysis of these studies involved comparing participants with the highest 20% schizotypy (cases) scores with those with the lowest 20% schizotypy scores (controls). This resulted in 33 cases and 33 controls in Study 1 and 14 cases and 14 controls in Study 2. This method was chosen to create a case-control design in order to be able to directly compare results from this study and other studies presented in this thesis.

4.4.2 MRI Data Acquisition

Individual anatomical MRIs (1-mm isotropic, T1-weighted (Cardiff: FSPGR, Nottingham: MPRAGE) were acquired using a 3.0 T MRI scanner (Cardiff: General Electric, Nottingham: Philips).

4.4.3 MEG Procedure and Data Acquisition

Identical 275-channel axial gradiometer CTF systems (VSM MedTech) were used at both centres to collect MEG data. For Study 1, participants were seated upright in the scanner. For Study 2 participants were orientated supine. Data were acquired at a sampling frequency of 1200Hz for the study 1 and 600Hz for study 2. Electromagnetic coils were placed at three fiduciary locations (bilateral pre-auricular and nasion) and their position relative to the MEG sensors was localised before and after each session. For study 1, MEG data was co-registered to the individual anatomical MRI of each participant by marking the positions of the fiducial coils on each MRI. For study 2, a 3D digitiser (Polhemus Inc., Vermont) was used to obtain a three-dimensional model of the participants' head shape, relative to the fiducial markers.

For both studies, participants completed one 5 minute, eyes open resting-state task.

Datasets were down-sampled to 600Hz (where required); band-pass filtered at 1-150Hz and segmented into 2 second epochs. Each epoch was then visually inspected for artefacts such as large muscle contractions or movement and if present, excluded from subsequent analysis. Datasets were filtered into the following bandwidths: Delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), low gamma (30–50 Hz) and high gamma (50-90 Hz).

4.4.4 Pipeline for Amplitude-Amplitude Coupling and Statistical Analyses

The analysis pipeline and statistical analyses used for the studies in this chapter have been described previously in Chapter 3. In summary, we used an AAL atlas-guided beamforming approach to define regions of interest and then cross-correlated amplitude envelopes to define connectivity between regions. A GMM approach was then used to define "signal" and "noise" connections and signal connections were then compared between groups. For further information see Chapter 3. Preliminary analysis was carried out initially on the whole cohort of 183 participants in Schizotypy Study 1 (Cardiff) for every frequency band. Using the GMM method outlined above, robust connections were only found in the alpha and beta frequency bands in this cohort and therefore only these frequency bands were used for subsequent analysis of datasets.

4.5 Results

4.5.1 Demographic and Clinical Information

The demographic and clinical assessment of case and control groups are shown in Table 4.1, where "Cases" refers to the high-SPQ group and "Controls" refers to the low-SPQ group. No significant difference in age or sex was found between groups. (Note: SPQ scores appear much higher for participants in Nottingham compared to Cardiff. This is because the SPQ questionnaire utilised in Nottingham required a Likert response whereas that in Cardiff required a binary response.)

	Study 1 (Cardiff)			Study 2 (Nottingham)		
Variable (mean+/- S.D.)	Control Group (n=33)	Case Group (n= 33)	p-value/ X^2	Control Group (n=14)	Case Group (n=14)	p-value/ X^2
Age (years)	22.9+/-3.34	24.45+/-4.3	0.11	37.03+/-11.78	38.92+/-11.13	0.67
Sex (M/F)	7M/26F	11M/22F	0.27	6M/8F	8M/6F	0.45
SPQ total score	1.79+/-1.05	27.79+/-6.2	*<0.01	106.57+/-15.71	217.93+/-16.13	*<0.01

Table 4.1 Demographic and clinical information
S.D = standard deviation, M=male, F= female, SPQ=Schizotypal personality questionairre (Note differing SPQ scores between Study 1 and 2 due to differing SPQ methodology.
*p<0.05 significant

4.5.2 Resting State Functional Connectivity Analysis: Uncorrected Analysis of Differences

Figure 4.1 shows grouped connectivity matrices for cases and controls and difference in mean connectivity for Schizotypy Studies 1 and 2 for all frequency bands. The left panel shows results for Study 1 and the right panel shows results for Study 2.

As per the results presented in Chapter 3, there is a hub region of increased connectivity (represented by a central block of orange on the connectivity matrices), particularly in the alpha and beta frequency bands that represents connectivity in the posterior parietal and occipital regions. This is evident for both cases and controls in Study 1 and 2. The difference in mean connectivity between cases and controls shows reduced connectivity in cases in the alpha frequency bands in both studies and in the beta (and somewhat theta) frequency band in Study 1. This is represented by blue regions and appears to be particularly prominent in the posterior parietal hub region previously discussed.

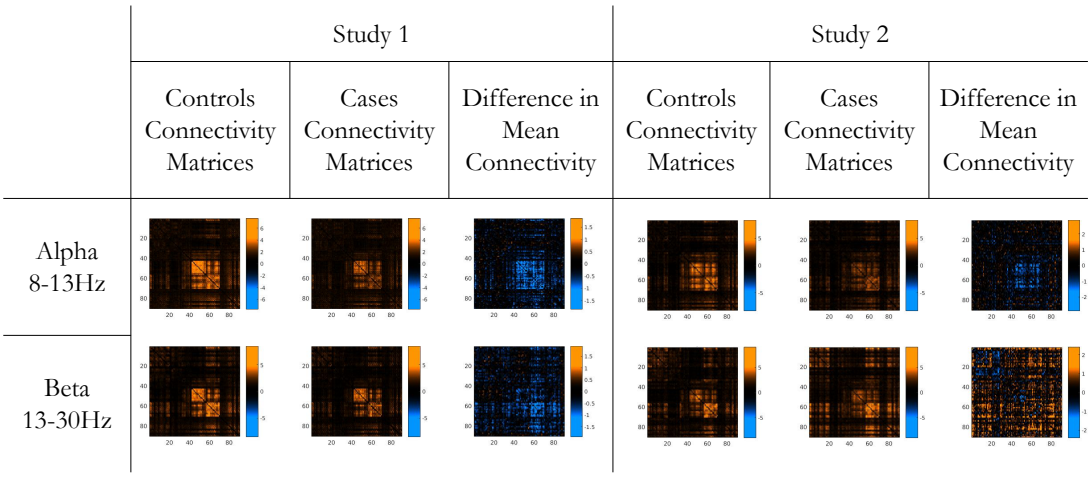


Figure 4.1: Schizotypy Study 1 and 2 Connectivity matrices.
Left panel = Study 1, Right panel = Study 2. Orange=higher connectivity. Blue=lower connectivity.

As seen in Figure 4.2, when taking a data masking approach, the results are similar, with the highest connectivity connections being mainly in posterior occipital and parietal regions as well as temporal, particularly for Study 1. For Study 2, the strongest connections are much more widespread. When calculating the difference in mean connectivity, there is a consistent reduction in connectivity in these occipital and parietal connections in both studies in the alpha frequency band and in Study 1 in the beta frequency band. In study 2, there is increased connectivity in the beta frequency band posteriorly and fronto-parietally.

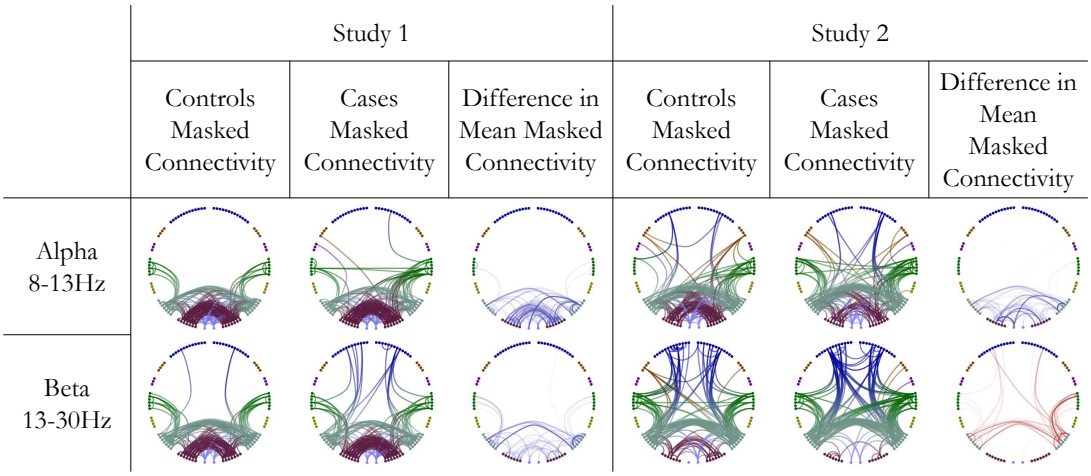


Figure 4.2: Schizotypy Study 1 and 2 Uncorrected Connectivity Maps.
Left panel = Study 1, Right panel = Study 2.
Masked connectivity refers to the connections with the highest 5% z-statistics, present 95% of the time in the bootstrapping procedure.
Controls Masked Connectivity = mean highest connections over all controls.
Cases Masked Connectivity = mean highest connections over all cases.
Difference in Mean Connectivity= Cases Masked Connectivity minus Controls Masked Connectivity.
Blue=lower connectivity in cases. Red = higher connectivity in cases.

4.5.3 GMM Analysis

When using a GMM approach, as seen in Figure 4.3, again, "signal" connections were found predominantly in occipital and parietal regions in the alpha frequency band. In the beta frequency band, signal connections were more widespread and in addition included fronto-parietal and temporo-parietal connections.

In both schizotypy studies, posterior (occipital and parietal) connections were consistently reduced in cases in the alpha frequency band. A corrected t-test resulted in the following connections being significantly reduced in cases:

Schizotypy Study 1:
Left Precuneus to Paracentral Lobule

Schizotypy Study 2:
Left Precuneus to Right Angular Gyrus
Left Precuneus to Right Cuneus

In the beta frequency band, reduced connectivity was found in Study 1 parieto-parietally. However, these differences did not reach statistical significance. In contrast, in Study 2, there were multiple connections showing increased connectivity. Again, none of these reached statistical significance.

Reductions in connectivity strength were found in both studies (Study 1, p=<0.01 (Right Superior Parietal (60), Study 2, p=0.0352 (Left Precuneus (67)) in the alpha frequency band. No significant differences in connectivity strength were found in the beta frequency band in either schizotypy study.

In the alpha frequency band, there was significantly reduced global connectivity in Schizotypy Study 1 (p=0.01908) in cases. Global alpha connectivity was non-significantly reduced in Schizotypy Study 2 (p=0.053713). No significant differences in global connectivity were found in the beta frequency band in either schizotypy study.

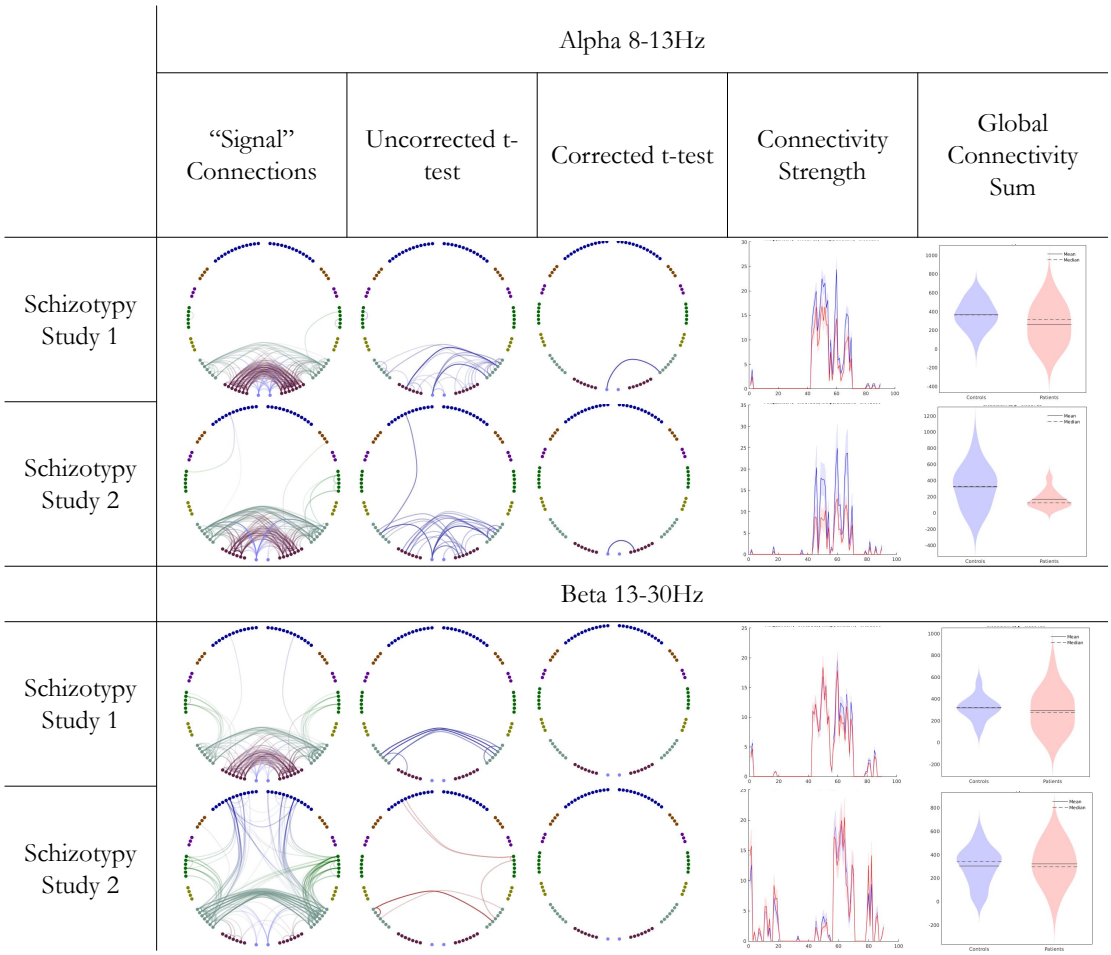


Figure 4.3: Schizotypy Study 1 and 2 GMM Analysis.
Signal connections= connections found to be in the "signal" Gaussian using GMM.
Uncorrected t-test= uncorrected t-test of signal connections between cases and controls.
Corrected t-test= corrected t-test of signal connections between cases and controls.
Red lines=increased connectivity. Blue lines= decreased connectivity in cases.
Significant Connections:
Alpha: Schizotypy Study 1: Left Precuneus to Paracentral Lobule
Schizotypy Study 2: Left Precuneus to Right Angular Gyrus, Left Precuneus to Right Cuneus
Connectivity Strength= Sum of every signal connection for each AAL node.
Horizontal axes=AAL node. Vertical axes=Connectivity strength.
Global Connectivity Sum= Violin plot of sum of all signal connections for cases and controls.
Red=Cases. Blue=Controls.

4.5.4 Correlation between Connectivity in Schizophrenia and Schizotypy

Further exploration and comparison of data presented in Chapter 3, Schizophrenia Study 1 and Schizotypy Study 1 presented in this chapter revealed interesting associations which are shown in Figure 4.4. I looked only at connections that were labelled as "valid" in both

Schizophrenia Study 1 (eyes open) *and* Schizotypy Study 1. T-tests of these connections between cases and controls in both studies were significantly correlated in the beta frequency band ($r=0.390078$, $p<0.01$), suggesting an association between beta connectivity in schizophrenia and beta connectivity in schizotypy.

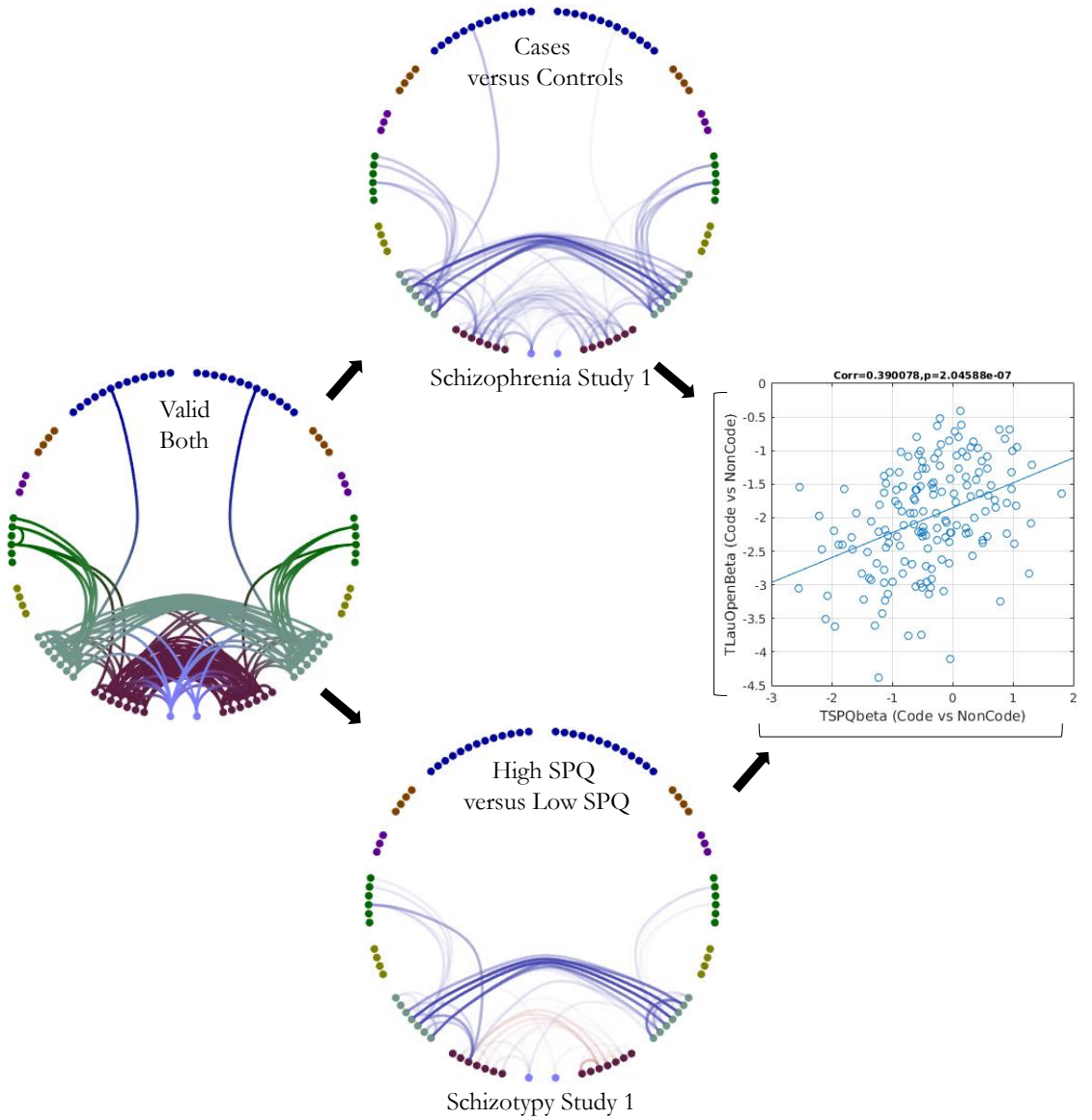


Figure 4.4: Beta band correlation between t-tests in Schizophrenia Study 1 and Schizotypy Study 1
Valid Both= Connections valid after GMM in both Schizophrenia Study 1 and Schizotypy Study 1
Cases versus Controls= t-test of valid connections between patients and controls in Schizophrenia Study 1
High SPQ versus Low SPQ= t-test of valid connections between cases and controls in Schizotypy Study 1
Correlation between t-tests of Schizophrenia Study 1 and Schizotypy Study 1

4.6 Discussion

Resting-state dysconnectivity has been repeatedly found in patients with schizophrenia (Yu et al., 2012). However, despite growing interest in the continuum hypothesis of schizophrenia, there are few studies exploring resting-state connectivity in schizophrenia spectrum disorders and none, to date, using MEG (Zhu et al., 2017, Wang et al., 2015, Zhang et al., 2014b, Lagioia et al., 2010).

A robust and repeatable metric (amplitude-amplitude coupling in MEG with spatial filtering of connections using Gaussian Mixture Modelling) was used to elucidate the neurobiological continuity between schizotypy and schizophrenia. Through this method, robust connections were identified in the alpha and beta frequency bands in a large cohort of healthy individuals. Using a GMM approach to identify only valid signal connections, I found reduced connectivity in two schizotypy studies in the alpha frequency band. In addition to global connectivity being reduced in both studies, connectivity of specific connections between the left precuneus and the right paracentral lobule, right angular gyrus and right cuneus were also reduced.

The precuneus forms an important part of the default mode network (DMN) along with the posterior cingulate cortex, medial prefrontal cortex and the bilateral temporo-parietal junction (Utevsky et al., 2014). The network shows increased activity at rest and reduced activity during externally orientated, task performance and is considered important for internally orientated cognition (Mantini and Vanduffel, 2013). Given its importance in cognition, multiple studies have sought to explore DMN connectivity in schizophrenia both at rest (Bluhm et al., 2007, Liu et al., 2012, Zhou et al., 2007) and during task performance (Whitfield-Gabrieli et al., 2009). More specifically, disrupted precuneus connectivity has been implicated in schizophrenia with one meta-analysis finding reduced resting-state precuneus connectivity in schizophrenia (Kuhn and Gallinat, 2013). Since then, other studies have also

found disrupted precuneus connectivity in schizophrenia. For example, Gong et al. (2014) found an association between functional connectivity of the precuneus and rare genetic variants in schizophrenia. Guo et al. (2014) also found disrupted resting-state functional connectivity in a parietal circuit including the precuneus in schizophrenia. These results suggest that DMN and specifically, precuneus connectivity is important in schizophrenia. The precuneus is believed to play a role in many cognitive functions including self-awareness and consciousness (Laureys et al., 2004), visuospatial imagery, episodic memory retrieval and self-processing (Reviewed by (Cavanna and Trimble, 2006), therefore providing a clear link with impairments seen in schizophrenia.

Studies exploring DMN, and specifically precuneus connectivity in schizophrenia spectrum disorders are much scarcer but along with our results, support neurobiological continuity between schizotypy and schizophrenia. For example, a study of resting-state functional connectivity in schizotypal personality disorder found reduced functional connectivity between bilateral precuneus and contralateral parahippocampus (Zhu et al., 2017). In addition, a study of structural changes in schizotypy found a correlation between precuneus grey matter volume and negative schizotypy (Nenadic et al., 2015).

In addition to significant differences between participants with high and low schizotypy scores in the alpha frequency band, I also found a significant correlation between t-test scores of differences between patients and controls in Schizophrenia Study 1 and t-test scores of differences between high and low schizotypy participants in Schizotypy Study 1 in the beta frequency band. This along with the results outlined above support biological continuity between schizophrenia and schizotypy.

4.7 Conclusion

In summary, I found reduced connectivity in two separate cohorts of healthy participants with high schizotypy scores in the alpha frequency band. Whilst this dysconnectivity was global, I also found reduced connections from the precuneus, an important part of the DMN that has been implicated in schizophrenia previously. Given this and the previous finding of dysconnectivity in schizophrenia in Chapter 3, this study supports the notion of a biological continuum between subclinical psychotic symptoms and diagnosable schizophrenia.

Chapter 5 The Impact of Disease Stage upon Resting State Connectivity in Schizophrenia; an MEG Study

5.1 Rationale

As previously discussed, evidence for functional dysconnectivity in schizophrenia is growing (Pettersson-Yeo et al., 2011). However, results from such studies are heterogeneous, with some finding increased connectivity, some finding reduced connectivity and some finding region dependent increases and decreases in connectivity. In Chapter 3, I found different patterns of connectivity changes in patients with schizophrenia in two similar studies using the same analysis pipeline, suggesting that differences between studies are not solely due to methodological heterogeneity. Different functional connectivity findings in schizophrenia could be due to the heterogeneity of the disorder, the stage of disease or other factors such as medication exposure or nicotine exposure. In this study, I sought to explore the impact of disease stage upon resting-state functional connectivity in schizophrenia.

5.2 Background

Schizophrenia is a heterogeneous disorder in terms of its presentation, course and prognosis. Throughout the course of the disorder, evidence suggests that progressive brain changes occur such as reductions in grey matter volume, cortical thinning and lateral ventricle enlargement (Olabi et al., 2011, Dietsche et al., 2017, Ho et al., 2003). Such changes can be evident even before illness onset. For example, it has been found that grey matter loss occurs even prior to illness onset in studies of Ultra High Risk (UHR) groups and that this

progresses as illness develops (Cannon et al., 2015). This is also reflected in studies of white matter changes (Carletti et al., 2012) and neurochemistry (Marsman et al., 2013).

As discussed previously, dysconnectivity has been implicated in the pathogenesis of schizophrenia and supported by numerous studies, including our own reported in Chapter 3. However, results from such studies are heterogeneous. Given that neurobiology appears to change throughout the course of schizophrenia, one contributing factor to the heterogeneity of functional connectivity research findings in the field may be the stage of the disorder studied.

When considering structural connectivity in schizophrenia, attempts have been made to explore the impact of stage of illness, age and course of onset in order to elucidate the progressive nature of such changes. Cross sectional studies looking at structural connectivity in both First Episode (FE) and chronic patients have found differences between the two. For example, Friedman et al. (2008) and Kong et al. (2011) both found significantly reduced FA in chronic patients but not in FE. White et al. (2011) found overall lower FA in patients which was more pronounced in chronic patients compared with FE. Other studies have found negative correlations between FA and illness duration (Carpenter et al., 2008). A meta-analysis of diffusor tensor imaging (DTI) in schizophrenia by Yang et al. (2017) found an inverse relationship between FA and age in patients with schizophrenia which is reflected in studies of FA in healthy ageing (Grieve et al., 2007). This replicates work by Rosenberger et al. (2008), who found an age-related decline in FA in patients with schizophrenia in certain fibre tracts which was not evident in healthy controls. In addition to studies comparing FE and chronic schizophrenia, studies have also compared UHR groups with FE. In a longitudinal study by Carletti et al. (2012), UHR patients that later went on to develop psychosis showed progressive reductions in FA over time. Taken together, such studies

suggest that there may be a progressive decline in structural connectivity throughout the course of schizophrenia.

Given such findings in the structural connectivity literature, many studies have attempted to explore stage specific changes in functional connectivity in schizophrenia. Functional connectivity findings in FE psychosis are mixed, with findings of variable (Yoon et al., 2015), reduced (Woodward et al., 2009, Benetti et al., 2009) and increased (Boksman et al., 2005) connectivity in patients. Of those studies exploring more chronic stages of schizophrenia using fMRI, the majority report reduced functional connectivity in patients (Pettersson-Yeo et al., 2011). Studies have attempted to elucidate this relationship between functional connectivity and stage of disease further by looking at different stages in the same study. For example, a meta-analysis by Li et al. (2017) showed predominantly frontal dysconnectivity (both increases and decreases) in first episode psychosis, becoming more widespread in "chronic stages", again suggesting some progression of functional dysconnectivity throughout the disorder.

Some authors postulate that the progressive changes seen in schizophrenia represent an "accelerated ageing" process (Kirkpatrick et al., 2008). Structural connectivity or white matter integrity has also been found to decline in healthy ageing and to be associated with cognitive ability and decreased functional connectivity in certain networks (Geerligs et al., 2015, Andrews-Hanna et al., 2007). Sheffield et al. (2016) found reduced pseudo-resting-state (whereby task-dependent signal is modelled and regressed out) functional connectivity in patients with schizophrenia in the cingulo-opercular and fronto-parietal networks. They found a stronger negative association between age and connectivity of these networks than in the healthy control group which the authors suggest supports accelerated ageing of these networks in schizophrenia.

5.3 Aims and Hypotheses

As discussed, a growing evidence base suggests that changes in brain structure and function may be progressive throughout the course of schizophrenia. Therefore, the grouping together of patients of all illness durations may have contributed to some of the heterogeneity in results seen between studies, such as those seen in Chapter 3.

I tested the hypothesis that resting-state functional connectivity differs depending on the stage of schizophrenia. I sought to explore whether functional dysconnectivity is present at very early stages of the illness and whether it is static and trait like or dynamic and progressive in nature. Given previous research findings in the field, I expected to see dysconnectivity present in early stages of schizophrenia but with reduced severity compared to later stages of the disorder. In this chapter, I will report data from the Medical Research Council Study of Psychosis and the Role of Inflammation, GABA and Glutamate (MRC SPRING). This is a cross-sectional, multi-centre study but here I only report data collected in Cardiff University. For consistency across studies, I will use the previously validated method of amplitude-amplitude coupling in resting-state MEG to explore dysconnectivity in different stages of schizophrenia.

5.4 Materials and Methods

5.4.1 Participants

The study was ethically approved in line with national practices (14/NW/0298). Participants gave written, informed consent prior to taking part.

The data used in this study were acquired as part of a multi-centre study of schizophrenia entitled MRC SPRING. The study was conducted at Cardiff University, The University of Nottingham and The University of Manchester. In this chapter, I will only present data

collected at Cardiff University by myself and Dr Loes Koelewijn. There were two arms of the study, one investigating recent onset psychosis and the other investigating established psychosis.

5.4.1.1 Recent Onset Psychosis Group

18 participants within 5 years of a first DSM-IV diagnosis of schizophrenia or schizophreniform disorder (13 males, 5 females; mean age: 23 +/-4.28, age range: 18-31, mean illness duration: 8.86 months) took part in the study. Participants had no exposure, discontinued or minimal exposure (<12 weeks) to antipsychotic medication.

5.4.1.2 Established Psychosis Group

20 participants with more than 10 years' history of a DSM-IV diagnosis of schizophrenia (17 males, 3 females; mean age: 39 +/-7.78, age range: 27-54, mean illness duration: 201.6 months) took part in the study. Participants had at least eight weeks' stable treatment prior to taking part in the study.

Cases were recruited through local Community Mental Health Teams (CMHTs), specialist Early Intervention for Psychosis Services and Clozapine clinics. Participants were diagnosed through clinical assessment and case note review followed by verification using a clinical research consensus diagnostic approach (involving myself and Dr James Walters) using DSM-IV criteria.

5.4.1.3 Healthy Control Groups

Both patient groups were matched to 10 healthy control participants according to age, sex and parental occupation. These were recruited locally through an online advert and the University noticeboard. The recent onset control group consisted of 7 males, 3 females; mean

age: 23 +/-3.03, age range: 18-26. The established control group consisted of 10 males, 2 females; mean age: 39 +/-7.78, age range: 29-54.

5.4.1.4 Inclusion and Exclusion Criteria

For all groups inclusion criteria were; male or female, aged 18 - 55 years, ability to understand and willing to give written informed consent and English as first language or fluent. Exclusion criteria for patient groups were; clinically significant neurological disorder, history of head injury with loss of consciousness >5 minutes, current harmful use of, or recent dependence on, psychoactive substances (excluding nicotine), contraindications for MR scanning (e.g. claustrophobia, pregnancy etc). Exclusion criteria for control groups were; personal history of psychosis or related disorder as determined by MINI-International Neuropsychiatric Interview (MINI), current or recent (within 2 years) presence of depressive symptoms or treatment with antidepressant medication, current use of any medication which may interfere with the study, first degree relative with a history of psychosis, clinically significant neurological disorder, history of head injury with loss of consciousness >5 minutes.

5.4.2 MRI Data Acquisition

Individual anatomical MRIs (1-mm isotropic, T1-weighted, FSPGR) were acquired using a 3.0 T MRI scanner (General Electric).

5.4.3 MEG Procedure and Data Acquisition

A 275-channel axial gradiometer CTF system (VSM MedTech) was used to collect MEG data. Participants were seated upright in the scanner. Data were acquired at a sampling frequency of 1200Hz. Electromagnetic coils were placed at three fiduciary locations (bilateral pre-auricular and nasion) and their position relative to the MEG sensors was localised before and after each session. MEG data was co-registered to the individual anatomical MRI of each participant by marking the positions of the fiducial coils on each MRI. Participants completed one 10 minute, eyes open resting-state task.

Datasets were down-sampled to 600Hz; band-pass filtered at 1-150Hz and segmented into 2-second epochs. Each epoch was then visually inspected for artefacts such as large muscle contractions or movement and if present, excluded from subsequent analysis. Datasets were filtered into the following bandwidths: Delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), low gamma (30–50 Hz) and high gamma (50-90 Hz).

5.4.4 Pipeline for Amplitude-Amplitude Coupling and Statistical Analyses

The analysis pipeline and statistical analyses used for the studies in this chapter have been described previously in Chapter 3. In summary, I used an AAL atlas-guided beamforming approach to define regions of interest and then cross-correlated amplitude envelopes to define connectivity between regions. A GMM approach was then used to define "signal" and "noise" connections and signal connections were then compared between groups. For further information see Chapter 3. Given previous analysis only finding robust connections in the alpha and beta frequency bands, only these frequency bands were used for subsequent analysis in this study.

5.4.5 Behavioural Assessment

The Positive and Negative Syndrome Scale (PANSS) semi-structured interview was administered to all participants to assess current psychopathology. The PANSS is a 30 item scale which allows assessment of positive, negative and general psychopathology (Kay et al., 1987). In addition, IQ was measured using the short form of the *Wechsler Adult Intelligence Scale* (Blyler et al., 2000) which included assessment of digit symbol, arithmetic, information and block design.

Medication history was obtained through thorough case note review (where possible), whereby medication name, start date, end date and maximum dosage was extracted. The defined daily dose of antipsychotic medication (DDD) was calculated using average maintenance doses stated by WHO (WHO, 2012). Lifetime DDD years of antipsychotic medication was calculated by adding cumulative DDD for each participant and dividing by 365.25 in line with Hulkko et al. (2017).

5.5 Results

5.5.1 Demographic and Clinical Information

The demographic and clinical assessment of case and control groups are shown in Table 5.1.

	Recent Onset			Established		
Variable (mean+/- S.D.)	Control Group (n=10)	Case Group (n=18)	p-value	Control Group (n=12)	Case Group (n=20)	p-value
Age (years)	23(3.03)	23(4.28)	0.53	41(8.45)	39(7.78)	0.45
Sex (M/F)	7/3	12/5	---	10/2	17/3	---
Illness Duration (months)		8.86(11.30)*			201.6(61.50)*	
FSIQ	97.80(17.53)	90.33(19.47)	0.32	100.50(18.90)	87.95(14.09)	0.04*
PANSS Positive		14.16(6.77)			10.95(2.64)	
PANSS Negative		11.83(5.78)			14.7(7.80)	
PANSS General		25.55(8.60)			24.3(6.09)	
PANSS Total		51.55(17.69)			49.95(13.88)	
PSP		73.88(10.78)*			62.5(12.51)*	
Current DDD		1.19(0.70)*			1.81(0.84)*	
Lifetime DDD Years		0.22(0.13)*			21.32(12.47)*	
Handedness (R/L)	8/2	16/2		10/2	19/1	
Socioeconomic group 1/2/3/4/5	4/2/1/3/0	6/2/5/1/4		6/2/0/4/0	9/2/1/2/6	

Table 5.1 Demographic and clinical information
S.D = standard deviation, M=male, F= female, *p<0.05 significant

5.5.2 Resting State Functional Connectivity Analysis: Uncorrected Analysis of Differences

Figure 5.1 shows grouped connectivity matrices for cases and controls and difference in mean connectivity for the Recent Onset Group and Established Group for the alpha and beta frequency bands. The left panel shows results for the recent onset group and the right panel shows results for the established group.

As per the results presented in Chapters 3 and 4, there is a hub region of increased connectivity (represented by a central block of orange on the connectivity matrices) that represents connectivity in the posterior parietal region. This is evident for both cases and controls for both the recent onset and established groups. The difference in mean

connectivity between cases and controls shows reduced connectivity in cases in both the alpha and beta frequency bands in both studies. This is represented by blue regions on the connectivity matrices and appears more pronounced for the established arm of the study. However, these changes appear fairly widespread and reflect a general reduction in connectivity throughout. This is likely to be due to signal to noise ratio differences.

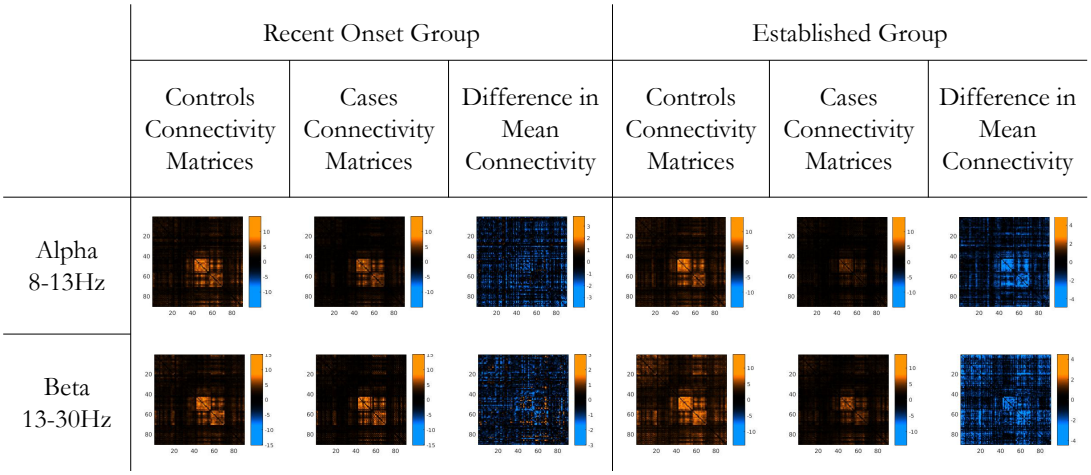


Figure 5.1: SPRING Connectivity matrices.
Left panel = Recent Onset Psychosis, Right panel = Established Psychosis. Orange=higher connectivity. Blue=lower connectivity.

As seen in Figure 5.2, when looking at the top 5% of connections, the results are similar, with the highest connectivity connections being mainly in posterior occipital and parietal regions as well as temporo-parietal and fronto-parietal connections. The strongest connections appear to be much more widespread for the Established Group study. When calculating the difference in mean connectivity, there is a consistent reduction in connectivity in these occipital and parietal connections in both studies in the alpha and beta frequency bands, although this is much more pronounced for the Established group study. For the recent onset group in the beta frequency band there is some evidence of increased connectivity in patients occipitally.

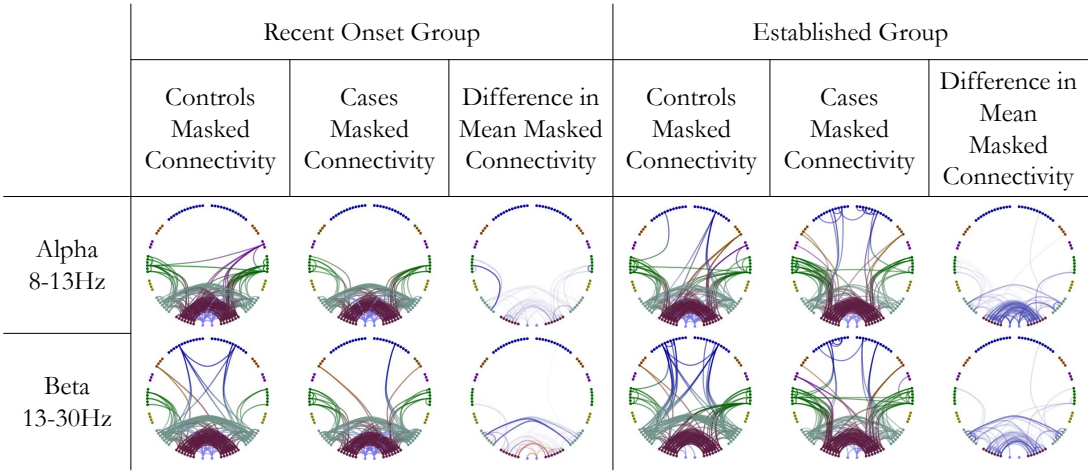


Figure 5.2: SPRING Uncorrected Connectivity Maps.
Left panel = Recent Onset Psychosis, Right panel = Established Psychosis.
Masked connectivity refers to the connections with the highest 5% z-statistics, present 95% of the time in the bootstrapping procedure.
Controls Masked Connectivity = mean highest connections over all controls.
Cases Masked Connectivity = mean highest connections over all cases.
Difference in Mean Connectivity= Cases Masked Connectivity minus Controls Masked Connectivity.
Blue=lower connectivity in cases. Red = higher connectivity in cases.

5.5.3 GMM Analysis

When using a GMM approach, as seen in Figure 5.3, "signal" connections were widespread but most pronounced in occipital and parietal regions in both the alpha and beta frequency bands.

Uncorrected t-tests revealed widespread reduced connectivity in patients in both the recent onset and established groups in both frequency bands. After corrected t-tests, none of these connections reached statistical significance. The GMM procedure has corrected for differences in SNR previously discussed and therefore only differences surviving correction are shown.

Reductions in connectivity strength (p=0.027) and global connectivity (p=0.013309) were found only in the established group in the alpha frequency band.

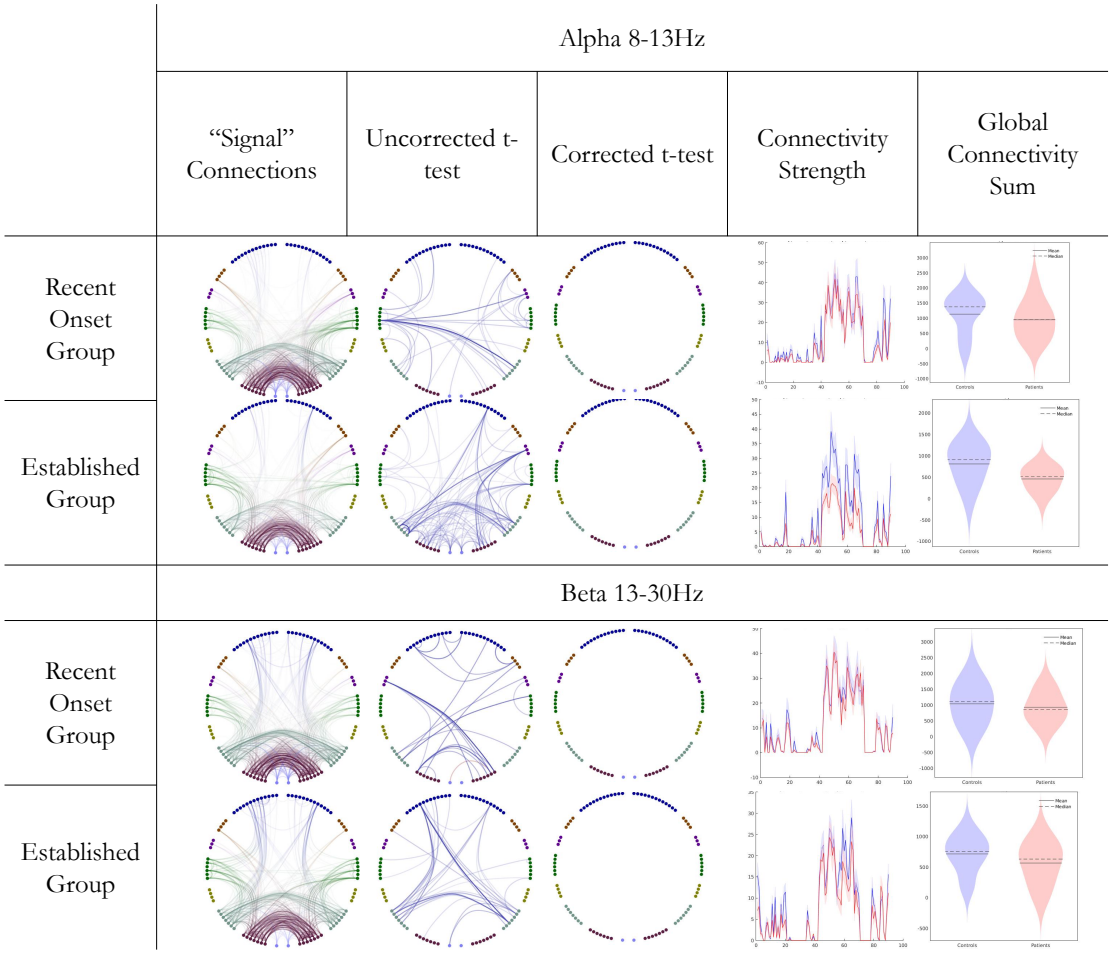


Figure 5.3: SPRING GMM Analysis.
Signal connections= connections found to be in the "signal" Gaussian using GMM.
Uncorrected t-test= uncorrected t-test of signal connections between cases and controls.
Corrected t-test= corrected t-test of signal connections between cases and controls.
Red lines=increased connectivity. Blue lines= decreased connectivity in cases.
Connectivity Strength= Sum of every signal connection for each AAL node.
Horizontal axes=AAL node. Vertical axes=Connectivity strength.
Global Connectivity Sum= Violin plot of sum of all signal connections for cases and controls.
Red=Cases. Blue=Controls.

5.5.4 Correlation with Clinical Scores

There were no statistically significant correlations between connectivity and age, PANSS scores (positive, negative, general and total), current DDD, lifetime DDD years or PSP for either group in either frequency band.

5.6 Discussion

There is a growing evidence base supporting resting-state dysconnectivity in patients with schizophrenia (Yu et al., 2012). However, results are inconsistent, particularly those using MEG (Bowyer et al., 2015, Kim et al., 2014, Hinkley et al., 2011). Evidence from studies of brain structure and function suggests that there are progressive neurobiological changes in schizophrenia throughout the course of the disorder. Therefore, one explanation for heterogeneity in resting-state findings may be heterogeneity in schizophrenia illness duration in study populations. I sought to explore the relationship between the chronicity of schizophrenia and functional connectivity using amplitude-amplitude coupling in MEG. Using this method, I analysed whole brain connectivity and found reductions in connectivity strength and global connectivity in patients with established schizophrenia in the alpha frequency band. I found no statistically significant differences in connectivity between patients with recent onset psychosis and healthy controls in either frequency band. This finding of a difference in connectivity between first episode and chronic stages of schizophrenia is consistent with structural connectivity findings (Friedman et al., 2008, Kong et al., 2011).

I did not find any correlations between connectivity and demographic or behavioural measures. Given hypotheses suggesting that schizophrenia represents a disorder of accelerated ageing (Sheffield et al., 2016), it is perhaps surprising that I did not find any correlations between age and connectivity. However, this may be because we looked at groups at two ends of the spectrum with the recent onset group having an average age of 23 and the established group having an average age of 39. I may have found an association between age and connectivity had I looked at patients across the span of the disorder. There may be non-linear effects of age that have not been captured by the sampling in this study.

Overall, these results support the hypothesis that there is a progressive decline in functional connectivity over the course of the disorder. However, there are inherent issues with making longitudinal conclusions based upon cross-sectional data. The conclusion that schizophrenia is a progressive condition and the differences between patients with early stage psychosis and established schizophrenia are due to disease progression is just one explanation for our findings. Other explanations include the following:

Firstly, in terms of their response to treatment, the groups studied may be very different. The "recent onset" group included any patient with schizophrenia or schizophreniform disorder within 12 weeks of treatment. Due to methods of recruitment used, the "established group" were predominantly a group of patients with treatment resistant schizophrenia, prescribed clozapine. 19 out of 20 patients were treated with clozapine.

It has been suggested that treatment resistant schizophrenia could be a different subtype of schizophrenia (Gillespie et al., 2017). As well as glutamatergic function (Mouchlianitis et al., 2016, Demjaha et al., 2014) and dopamine synthesis capacity (Demjaha et al., 2012) being different in treatment resistant schizophrenia, there is also reduced grey matter volume (Anderson et al., 2015) when compared to treatment responders. It may therefore follow

that patients with treatment resistant schizophrenia show different changes in functional connectivity when compared to treatment responders (Paul and Sharfman, 2016). This is supported by a study by White et al. (2016) who found reduced connectivity between the ventral striatum and the substantia nigra and between the dorsocaudal putamen and the thalamus and increased connectivity between dorsal caudate and medial prefrontal cortex in treatment resistant patients compared to responders.

A systematic review of longitudinal outcomes in schizophrenia revealed that while there is around a 40% remission rate, approximately 25% of patients have a "poor outcome" (Menezes et al., 2006). Given this heterogeneous prognosis, our recent onset group is likely to be mixed according to response to treatment and hence whether they will go on to be considered as having treatment resistant schizophrenia or even established schizophrenia.

Therefore, if our recent onset group consisted of patients with mixed prognosis, they may not be representative of established patients who go on to have treatment resistant schizophrenia. This difference between prognosis in the groups may account for differences in functional connectivity.

Secondly, an issue with most studies that divide patients according to illness duration is antipsychotic medication exposure. Those with a longer illness duration are likely to have been exposed to medication for longer. In our cohorts, there were statistically significant differences between recent onset and established groups according to current DDD and lifetime DDD years. Differences in connectivity between early and late stages of the disorder may therefore be explained by antipsychotic medication exposure. Several studies have explored functional connectivity in schizophrenia pre-and post-treatment with antipsychotics using resting state paradigms (Hadley et al., 2014, Sarpal et al., 2015, Kraguljac et al., 2016c). These studies have found normalisation of dysconnectivity in schizophrenia

after antipsychotic treatment both in the short term (Kraguljac et al., 2016c, Hadley et al., 2014) and longer term (1 year) (Anticevic et al., 2015b). It therefore appears that antipsychotic medications have an impact upon functional connectivity either directly or indirectly. Unfortunately, to my knowledge, no studies have explored the long term (>10 years) effects of antipsychotic exposure upon connectivity in a longitudinal design. In this study, medication exposure is unlikely to be a significant confounding factor as I did not find any statistically significant correlation between connectivity and medication exposure in either group. However, it is important to consider that this study has weak statistical power since there were only 10 healthy controls for each group of cases. This could be considered a limitation of the study.

5.7 Conclusion

In summary, I found reduced connectivity in patients with established schizophrenia in the alpha frequency band but not those within the early stages of psychosis. These results add further support to the dysconnectivity hypothesis of schizophrenia but also may provide support to the hypothesis that neurobiological changes in schizophrenia are progressive throughout the disorder. However, other explanations for such findings including medication exposure have been discussed and should be considered.

Chapter 6 An Investigation of the Modulation of MEG Resting-State Connectivity by Acute Administration of Ketamine

6.1 Rationale

Glutamate dysfunction has been implicated in the neuropathology of schizophrenia. This hypothesis suggests that disruptions to the neurotransmitter glutamate cause excitotoxicity, dopamine dysfunction and resultant psychopathology seen in schizophrenia. This was initially supported by work finding that NMDA receptor antagonists such as ketamine produce symptoms similar to those seen in schizophrenia and subsequently, ketamine has been adopted as a model of schizophrenia. There is a growing evidence base suggesting that functional dysconnectivity contributes to the psychopathology of schizophrenia. This is also supported by findings from studies reported in previous chapters of this thesis. In this chapter, I sought to explore the link between dysconnectivity and the glutamate hypothesis through exploring the impact of ketamine (NMDA antagonist) upon connectivity in a healthy group of participants.

6.2 Background

Glutamate is the primary excitatory neurotransmitter in the central nervous system (Rothman et al., 2003) and acts upon four different classes of receptor, the most significant in schizophrenia being the ionotropic NMDA receptor. The NMDAR is important in synaptic plasticity, cortical development, learning and working memory (Collingridge et al., 2013), all of which are relevant in the psychopathology of schizophrenia.

Glutamate was initially implicated in the pathogenesis of schizophrenia due to the psychotogenic effects of dissociative anaesthetics such as ketamine which act by blocking the NMDAR. NMDAR antagonists block NMDA receptors on GABAergic interneurons resulting in excess glutamate release (Stone et al., 2012)and excitotoxicity. This has been postulated as a model of schizophrenia since such drugs exacerbate symptoms in patients with schizophrenia (Lahti et al., 1995) and mimic positive, negative and cognitive symptoms of schizophrenia in healthy controls (Adler et al., 1999, Krystal et al., 2005). Therefore, unlike the dopaminergic hypothesis of schizophrenia, the glutamatergic hypothesis explains not only the positive symptoms of the condition but also the negative and cognitive symptoms. It may also explain the differences in response to traditional antipsychotic medications seen in patients (Egerton et al., 2012).

The involvement of glutamate pathways in the aetiology of schizophrenia is supported by proteomic and genomic studies. Pathogenic CNVs in schizophrenia have been found to converge upon genes involved in glutamatergic and GABAergic neurotransmission (Pocklington et al., 2014) supporting hypotheses of the aetiology of schizophrenia implicating these neurotransmitter systems.

In addition, in vivo studies using Magnetic Resonance Spectroscopy (MRS) have found abnormal glutamate levels in schizophrenia (Marsman et al., 2013). Since glutamate is known to have excitotoxic effects when present in excess (Lau and Tymianski, 2010) it has been postulated that in the early stages of schizophrenia hyper-glutamatergia leads to excitotoxicity (Plitman et al., 2014). This is supported by a review of MRS studies by Poels et al. (2014) who found an increase in glutamatergic levels in the medial prefrontal cortex in early stage drug naïve patients with schizophrenia compared to controls. A meta-analysis by Marsman et al. (2013) found that glutamate reduces with age in patients with schizophrenia suggesting possible neurochemical differences between early and later stages of the disorder.

As previously discussed, it is postulated that symptoms of schizophrenia result from abnormal communication within and between cortical networks (Fornito et al., 2012). This is supported by multiple studies including work presented in this thesis. Synchronised oscillatory activity (as measured non-invasively in humans using magnetoencephalography - MEG) is required for such communication. This oscillatory activity is dependent upon the interaction between excitatory (glutamatergic) and inhibitory (GABAergic interneuron) activity (Uhlhaas et al., 2008). Given this, and findings of dysconnectivity in schizophrenia presented in previous chapters, we may therefore expect the administration of ketamine, a model of schizophrenia, to result in dysconnectivity.

Several studies have explored the effects of ketamine upon functional connectivity through the acute administration of low dose ketamine in healthy participants. Most, aside from Rivolta et al. (2015) and Muthukumaraswamy et al. (2015) who used MEG, explored this using fMRI. Results have been mixed, with some finding increased connectivity following ketamine administration (Rivolta et al., 2015, Hoflich et al., 2015) and others finding reduced connectivity (Kraguljac et al., 2016a, Scheidegger et al., 2012). Interestingly, Driesen et al. (2013b) found decreased connectivity during a working memory task but increased connectivity at rest (Driesen et al., 2013a) following ketamine. This suggests that its effects upon functional connectivity may be state dependent. Effects may also relate to the amount or duration of ketamine use with a more naturalistic study finding reduced resting-state functional connectivity in chronic ketamine users (Liao et al., 2016).

When considering the link between the neurological impact of ketamine and the neuropathology of schizophrenia, Anticevic et al. (2015a) found increased connectivity following ketamine administration and in a group of patients with early schizophrenia but reduced functional connectivity in chronic schizophrenia. The authors therefore suggest that

ketamine is a better model for the early stages of schizophrenia than the late stages. This would also fit with the previously discussed MRS findings in schizophrenia.

6.3 Aims and Hypotheses

Given the accumulating evidence for dysconnectivity in schizophrenia, (supported by findings in Chapter 3 and 5), as well as the glutamate hypothesis of schizophrenia, I sought to explore functional connectivity following acute ketamine administration. Through investigating functional connectivity in healthy individuals given ketamine, I attempted to investigate the link between this postulated model of schizophrenia and observations in the condition itself.

Whilst functional connectivity findings following acute ketamine administration are mixed, increased resting-state connectivity has been found in multiple studies (Anticevic et al., 2015a, Driesen et al., 2013a, Rivolta et al., 2015, Hoflich et al., 2015). Since I found reduced connectivity in chronic stages of schizophrenia and it has been postulated that ketamine models earlier stages of the disorder (Anticevic et al., 2015a), I expect to find connectivity with ketamine to differ from that found in chronic schizophrenia.

The data in this study was generated by, and has previously been published by, Muthukumaraswamy et al. (2015) but in this chapter, I will use the analysis pipeline used throughout this thesis in order to make direct comparison with our previously reported data in schizophrenia.

6.4 Materials and Methods

6.4.1 Participants

Eighteen healthy young men with a mean age of 25.05 (age range 19-36) took part in the study. The study was ethically approved by a UK National Health Service research ethics committee. Participants were aged between 18-45, non-smokers, American Society of Anaesthesiologists Physical Status 1, body mass index of 18-30kg/m2. Participants gave written, informed consent prior to taking part. The following exclusion criteria were applied: any current or previous psychiatric disorder (determined by the MINI), current recreational or prescription drug use, MEG/MRI contraindications, needle phobia.

6.4.2 MRI Data Acquisition

Individual anatomical MRIs (1-mm isotropic, T1-weighted FSPGR) were acquired using a 3.0 T MRI scanner (General Electric).

6.4.3 MEG Procedure and Data Acquisition

For further details regarding data acquisition, see Muthukumaraswamy et al. (2015).

Over two separate days, participants underwent two MEG scans, one with a placebo saline infusion and the other using ketamine. Infusion was commenced following the recording of 5 minutes resting-state MEG. An initial bolus of 0.25mg/kg was delivered over 1 minute, followed by maintenance infusion at 0.375mg/kg/h. At the start of the infusion, 10 minutes of eyes-open resting-state MEG was commenced.

A 275-channel axial gradiometer CTF system (VSM MedTech) was used to collect MEG data. Participants were orientated supine in the scanner. A probe over the left index finger monitored pulse rate and blood oxygenation level continuously. The intravenous cannula for infusion was located on the back of the left wrist. In case of emergency, a nasal cannula attached to medical oxygen was worn.

Data were acquired at a sampling frequency of 1200Hz. Electromagnetic coils were placed at three fiduciary locations (bilateral pre-auricular and nasion) and their position relative to the MEG sensors was localised before and after each session. MEG data was co-registered to the individual anatomical MRI of each participant by marking the positions of the fiducial coils on each MRI.

Datasets were down-sampled to 600Hz; band-pass filtered at 1-150Hz and segmented into 2 second epochs. Each epoch was then visually inspected for artefacts such as large muscle contractions or movement and if present, excluded from subsequent analysis. Datasets were filtered into the following bandwidths: Delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), low gamma (30–50 Hz) and high gamma (50-90 Hz).

6.4.4 Pipeline for Amplitude-Amplitude Coupling and Statistical Analyses

The analysis pipeline and statistical analyses used for the studies in this chapter have been described in detail previously in Chapter 3. In summary, I used an AAL atlas-guided beamforming approach to define regions of interest and then cross-correlated amplitude envelopes to define connectivity between regions. A GMM approach was then used to define "signal" and "noise" connections and signal connections were then compared between groups. For further information see Chapter 3. Given that in previous chapters, robust

connections were only found in the alpha and beta frequency bands, further analysis in this chapter will focus on these frequency bands only.

6.5 Results

6.5.1 Resting State Functional Connectivity: Uncorrected Analysis of Differences

Figure 6.1 shows grouped connectivity matrices for placebo and ketamine and difference in mean connectivity between the two in the alpha and beta frequency bands.

As per the results presented in Chapters 3, 4 and 5, there is a hub region of increased connectivity (represented by a central block of orange on the connectivity matrices) that represents connectivity in the posterior occipital and parietal region. This is evident in both placebo and ketamine conditions. In the placebo condition, there also appear to be more widespread regions of increased connectivity. The difference in mean connectivity between placebo and ketamine shows widespread reduced connectivity with ketamine in both frequency bands (represented by blue regions on the connectivity matrices). There appear to some small central (posterior parietal) regions of increased connectivity with ketamine also.

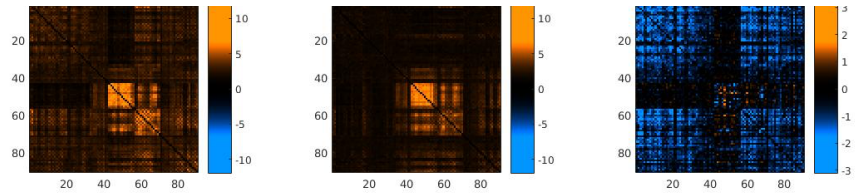
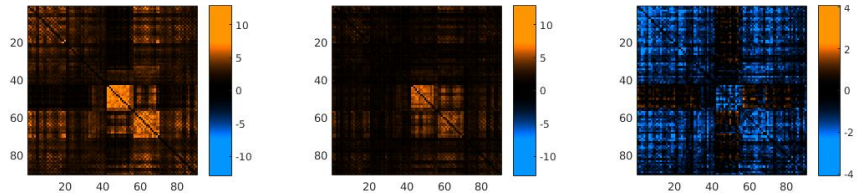
	Placebo Connectivity Matrices	Ketamine Connectivity Matrices	Difference in Mean Connectivity
Alpha 8-13Hz			
Beta 13-30Hz			

Figure 6.1: Ketamine Study Connectivity matrices.
Orange=higher connectivity. Blue=lower connectivity.

Figure 6.2 shows results following a data masking approach. The highest connectivity connections are again, mainly posterior but also bilateral temporo-parietal and fronto-parietal. Differences in mean connectivity between placebo and ketamine show posterior reductions in connectivity following ketamine administration. In the alpha frequency band, there appear to be some connections that are increased with ketamine.

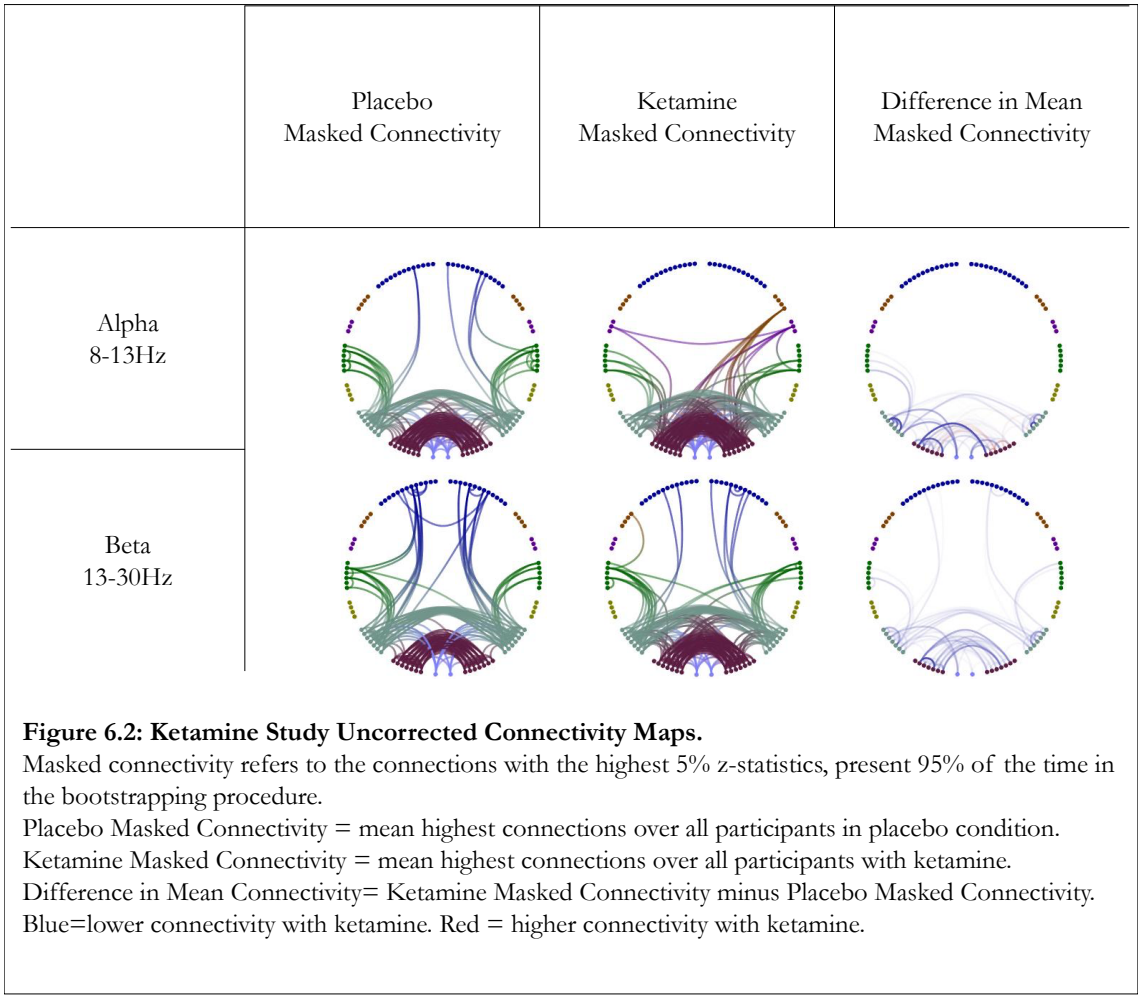


Figure 6.2: Ketamine Study Uncorrected Connectivity Maps.
Masked connectivity refers to the connections with the highest 5% z-statistics, present 95% of the time in the bootstrapping procedure.
Placebo Masked Connectivity = mean highest connections over all participants in placebo condition.
Ketamine Masked Connectivity = mean highest connections over all participants with ketamine.
Difference in Mean Connectivity= Ketamine Masked Connectivity minus Placebo Masked Connectivity.
Blue=lower connectivity with ketamine. Red = higher connectivity with ketamine.

6.5.2 GMM Analysis

The results from the GMM analysis can be seen in Figure 6.3. In the alpha frequency band, "signal" connections were found predominantly in occipital, parietal and temporal regions. Signal connections were more widespread in the beta frequency band with prominent fronto-parietal connections bilaterally.

In the alpha frequency band, an uncorrected t-test of differences between placebo and ketamine conditions shows increased connectivity with Ketamine, predominantly occipitally and parietally. Following correction for multiple comparisons, the following connections

were significantly stronger (0.005) in the alpha frequency band in the ketamine condition than placebo:

Right lingual to right mid occipital

Right lingual to right inferior occipital

In the beta frequency band, an uncorrected t-test showed more widespread, varied differences between placebo and ketamine. Following correction for multiple comparisons, there were no statistically significant differences between placebo and ketamine in this frequency band.

Connectivity strength of the right lingual AAL node was increased with ketamine in the alpha frequency band (0.0178). There were no statistically significant differences in connectivity strength between placebo and ketamine in the beta frequency band. Global connectivity appeared to be increased in the alpha frequency band with ketamine but this was not statistically significant ($p=0.1$). There were no differences in global connectivity in the beta frequency band.

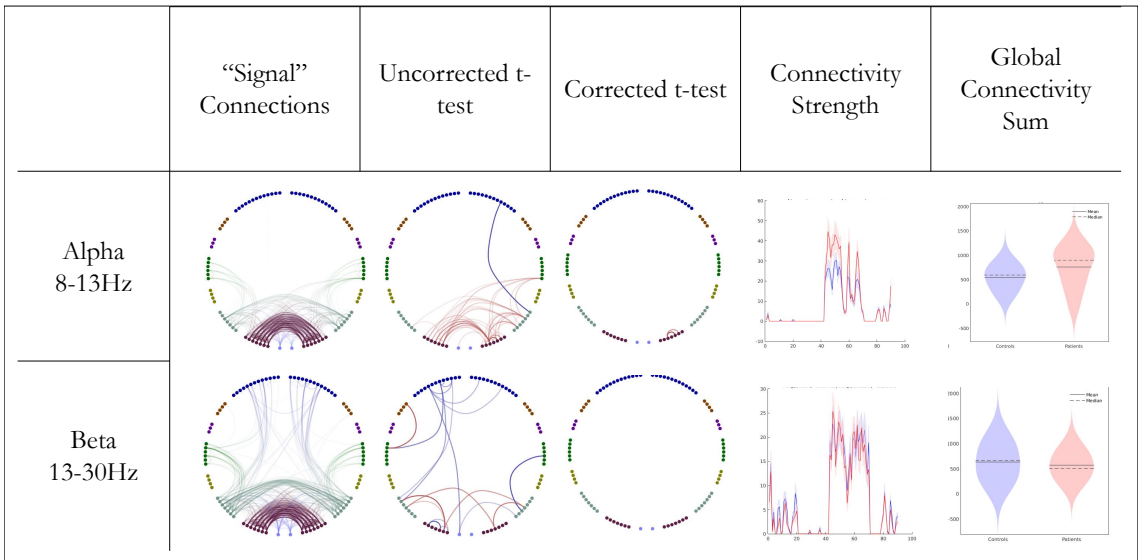


Figure 6.3: Ketamine Study GMM Analysis.
Signal connections= connections found to be in the "signal" Gaussian using GMM.
Uncorrected t-test= uncorrected t-test of signal connections between placebo and ketamine.
Corrected t-test= corrected t-test of signal connections between placebo and ketamine.
Red lines=increased connectivity. Blue lines= decreased connectivity with ketamine.
Significant Connections:
Alpha: Right lingual to Right Mid Occipital
Right lingual to Right Inferior Occipital
Connectivity Strength= Sum of every signal connection for each AAL node.
Horizontal axes=AAL node. Vertical axes=Connectivity strength.
Global Connectivity Sum= Violin plot of sum of all signal connections for placebo and ketamine.
Red=Ketamine. Blue=Placebo.

6.6 Discussion

Over many years, the glutamate hypothesis of schizophrenia has become a leading aetiological hypothesis of the disorder. This is in part due to the observed effects of NMDA receptor antagonist drugs such as ketamine which mimic the symptoms of schizophrenia (Adler et al., 1999). As a result, ketamine has now become a model of schizophrenia (Frohlich and Van Horn, 2014).

Given our previously reported findings of dysconnectivity in schizophrenia, in this chapter, I explored the effects of ketamine upon connectivity in a healthy group of young men. The aim was to add evidence to the ketamine model of schizophrenia and further develop links between the glutamate hypothesis and the dysconnectivity hypothesis. Using the same method used in previous chapters (amplitude-amplitude coupling in MEG), using a GMM

procedure, I found increased connectivity following acute ketamine administration in the alpha frequency band. The connections that were found to be significantly increased following ketamine administration were occipital, from the right lingual to right mid occipital and right inferior occipital. Connectivity strength was also significantly increased in the alpha frequency band following ketamine administration.

Results of this study reflect previous findings of increased connectivity following ketamine administration (Hoflich et al., 2015, Rivolta et al., 2015, Driesen et al., 2013a, Anticevic et al., 2015a). Of these studies, the majority used fMRI to explore functional connectivity, the exception being a resting-state MEG study by Rivolta et al. (2015). Using transfer entropy, Rivolta et al. (2015) found thalamo-cortical hyper-connectivity involving the visual cortex, similar to present findings using amplitude envelope correlation in MEG.

Interestingly, using the same dataset but an ICA based analysis method, Muthukumaraswamy et al. (2015) found reduced activity in bilateral parietal and motor networks in the beta frequency band and an occipital network in the alpha frequency band. (Since ICA is a measure of network activity rather than node-node connectivity this apparent decrease as reported by Muthukumaraswamy et al. (2015) may be due to decreased power in the alpha and beta frequency bands.) This suggests that choice of analytic pipeline can have a significant outcome upon results and may explain some of the heterogeneity seen in studies exploring functional connectivity with ketamine. This is also evident within this analysis where initial simple analysis, as seen in Figure 6.1, appears to show widespread reduced connectivity with Ketamine, whereas using a GMM approach (Figure 6.3) resulted in mostly increased connectivity, particularly in the alpha frequency band. This is because, Ketamine generates widespread differences across the connectivity matrix in almost all nodes. The GMM procedure removes noise and "corrects" for this by re-centering the null distributions for each condition. This then enables us to see apparent local increases in connectivity with

Ketamine. (Appendix 1 further discusses the benefits of the GMM approach using analysis from this study.)

In addition, the study design can have an impact, with a task based study by Driesen et al. (2013b) finding hypo-connectivity with ketamine and a resting-state study by Driesen et al. (2013a) finding hyper-connectivity with ketamine.

Using the same robust and repeatable analysis pipeline throughout this thesis has the benefit of allowing us to compare results across chapters. The finding of hypo-connectivity in schizophrenia (presented in Chapter 5), particularly the later stages of schizophrenia, fits with the hypothesis that acute ketamine administration does not model the later stages of schizophrenia well. Whilst in Chapter 5, I did not find statistically significant increases in connectivity in early stages of illness, other studies have found similarities between connectivity in early schizophrenia and following ketamine administration. For example, Anticevic et al. (2015a) explored functional connectivity in acute ketamine administration, high risk, early and late stages of schizophrenia. They found increased connectivity in healthy controls given ketamine as well as high risk and early schizophrenia but reduced connectivity in the group with chronic schizophrenia. They conclude that these findings suggest ketamine models earlier stages of schizophrenia better than later stages. This is supported by other studies such as Liao et al. (2016) who found hypo-connectivity in chronic ketamine users suggesting acute versus chronic NMDAR antagonism may have differential effects upon functional connectivity. Animal studies also support this, with a study by Ahnaou et al. (2017) finding increased phase amplitude coupling following acute administration of ketamine but decreased coherence with chronic ketamine administration.

6.7 Conclusion

In conclusion, I found increased occipital connectivity in the alpha frequency band in a group of healthy males following acute administration of ketamine- an NMDA receptor antagonist. Given our previous findings of reduced connectivity in patients with schizophrenia, particularly the more chronic stages, these results support the hypothesis that ketamine does not model the later stages of schizophrenia well. Taken together with previous research, ketamine may model the earlier stages of the disorder better than the later stages.

Chapter 7 Summarising Cohort Differences in Functional Connectivity across all Studies

7.1 Rationale

Throughout this thesis, I have presented functional connectivity findings from six different studies in order to explore not only the dysconnectivity hypothesis of schizophrenia but how it links with other hypotheses such as the glutamate hypothesis and the continuum hypothesis of schizophrenia. These studies have given interesting, and on the whole converging results but in this chapter, I seek to bring the studies together in order to more directly compare the results and to quantify effect magnitudes across these studies.

7.2 Background, Aims and Hypotheses

As discussed throughout the thesis, there are multiple hypotheses relating to schizophrenia, not all of which are mutually exclusive. In Chapter 3, I sought to explore the dysconnectivity hypothesis of schizophrenia in two studies of patients with non-duration or severity specific schizophrenia. In Chapter 4, I explored functional connectivity in the context of the continuum hypothesis of schizophrenia which suggests that schizophrenia exists on a continuum with subclinical psychotic symptoms in the healthy population. In Chapter 5, I explored the dysconnectivity hypothesis of schizophrenia in more detail, comparing earlier stages to later stages of the disorder. Finally, in the most recent chapter, Chapter 6, I looked at functional connectivity following acute ketamine administration in order to explore the link between the dysconnectivity hypothesis and the glutamate hypothesis of schizophrenia. All of these studies have resulted in a somewhat converging picture, however, in this chapter, I will explore this further by using an analysis technique that will allow a more direct

comparison of results from these studies. My aim will be to develop a cohesive picture of dysconnectivity in schizophrenia, schizotypy and with ketamine (a model of schizophrenia).

7.3 Materials and Methods

The studies included in this chapter have been described in detail in previous chapters but a brief description of each follows:

Study 1: Schizophrenia (Cardiff), eyes-open and eyes-closed resting-state conditions.

This study included 28 patients with non-duration or severity specific schizophrenia. However, overall, the group had established schizophrenia with an average age of 44.6. All, aside from one were treated with antipsychotic medication.

Study 2: Schizophrenia (Nottingham: MISP), eyes-open resting-state condition.

This study also included 28 patients with non-duration or severity specific schizophrenia. However, in comparison to the first study, patients were younger and more varied in their duration of illness with an average age of 26.7.

Study 3: Schizotypy (Cardiff), eyes-open resting-state condition.

In this study, 183 healthy individuals were administered the Schizotypal Personality Questionnaire (SPQ). Of these participants, individuals were divided into the highest and lowest 20% according to their scores on the SPQ. The high and low schizotypy groups were then compared to each other in further analysis.

Study 4: Schizotypy (Nottingham), eyes-open resting-state condition.

Similar methodology as above was used for this study of 70 healthy individuals.

Study 5: Recent Onset Schizophrenia (SPRING), eyes-open resting-state condition.

This study included 18 patients with schizophreniform disorder or schizophrenia within 12 weeks of treatment with antipsychotic medication.

Study 6: Established Schizophrenia (SPRING), eyes-open resting-state condition.

20 patients with schizophrenia of more than 10 years' duration were included in this study. All except one were prescribed Clozapine and can therefore be considered to be treatment resistant.

Study 7: Ketamine, eyes-open resting-state condition.

In this study, resting-state MEG was analysed following placebo and ketamine administration in 18 healthy young men.

7.3.1 Data Acquisition and Statistical Analyses

The MRI and MEG procedures for data acquisition and analysis have been described in detail in previous chapters. The pipeline for amplitude-amplitude coupling and statistical analysis have also been described previously. Following on from the GMM approach used in all previous analyses, we limited analysis to look at only those "signal" connections present, in all studies, at a threshold of 50%. To be clear, a node-node connection was only analysed if it was identified in every separate GMM analysis as having a group-mean probability of being valid "signal">50%. This left a more limited number of connections to take forward for subsequent analysis.

For these "group valid" connections I then assess the relative magnitude of cohort effects by averaging the t-statistic across all these connections to give one summary statistic.

7.3.1.1 Meta-Analysis

Given that I have completed several similar studies, I went on to use a meta-analytic approach to pool all of the data and look for group differences. For this analysis, I excluded the Schizophrenia Study 1 eyes-closed data since this would then include the same cohort of individuals twice and all other studies were completed with eyes-open. Pooled mean difference and heterogeneity were analysed using the Review Manager programme, version 5.3 (Manager, 2014). The mean and standard deviation (of all connections that were valid in all studies) for each group for each study was used to calculate a standardised mean difference. This is the difference in mean outcome between groups divided by the standard deviation of outcome among participants. A weighted average of pooled data was then calculated by summing mean difference estimates multiplied by weights (where weight=1/(standard error^2 + inter-trial variance) and dividing this by the sum of weights for all studies. The standard error of pooled effect size was then used to derive a confidence interval and p value to estimate the strength of evidence against the null hypothesis of no pooled effect. I used a random effects model (DerSimonian and Laird, 1986) whereby standard errors are adjusted to take account of heterogeneity. This is used when different studies are estimating different but related effects and takes into account both individual and between study variance (Deeks et al., 2008).

7.4 Results

Figure 7.1 below shows summarised alpha connectivity cohort-differences for all studies. Figure 7.1A shows a connectivity map of those 62 connections that were valid (mean(p)>50%) in *all* studies. Figure 7.1B shows t-test statistics for each of these valid connections in each study. Overall, there appears to be reduced posterior alpha connectivity

in these connections in most of the studies, with Study 2 (Nottingham MISP) appearing to have weaker connectivity reductions in patients than the other studies. In Study 7 (SPRING Recent Onset) connectivity appears much more mixed, with both reduced and increased connectivity in patients in these connections, but with weaker effect sizes. Study 5 (Ketamine) diverges significantly from the other studies with connectivity in these valid connections being increased with ketamine compared to placebo. Figure 7.1C shows boxplots of mean t-statistics across all of the valid connections for each study. These results echo those seen in Figure 7.1B, with connectivity in Study 5 (Ketamine) being increased and all other studies aside from Study 7 (SPRING Recent Onset) having reduced connectivity. In study 7, connectivity is slightly increased in cases but almost at zero (mean t-statistic) with no difference between cases and controls. Similar effect sizes are seen across the other studies showing reduced connectivity, except for Study 2 (Nottingham MISP) where the effects are much weaker. Figure 7.1D shows the ranked t-statistics for each connection for all studies. Again, results are similar, with connectivity in Study 5 (Ketamine) being increased with ketamine for most connections. In Study 7 (SPRING Recent Onset), the line crosses zero with some connections being increased and some being reduced in cases. Connectivity is mostly reduced in cases in Study 2 (Nottingham MISP) but with a weaker effect and again, the line crossing zero suggesting increased connectivity in some connections.

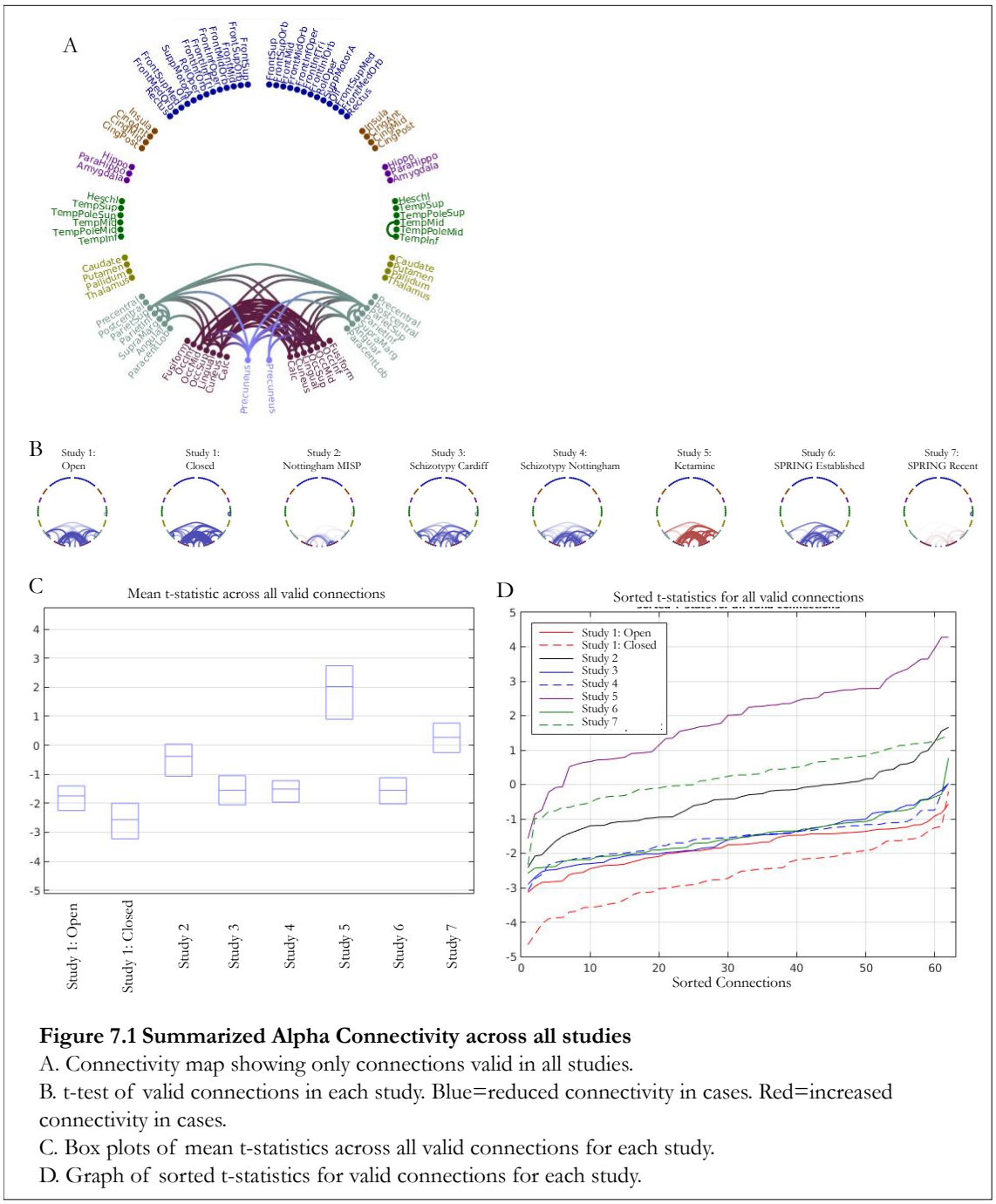


Figure 7.1 Summarized Alpha Connectivity across all studies
A. Connectivity map showing only connections valid in all studies.
B. t-test of valid connections in each study. Blue=reduced connectivity in cases. Red=increased connectivity in cases.
C. Box plots of mean t-statistics across all valid connections for each study.
D. Graph of sorted t-statistics for valid connections for each study.

Figure 7.2 below shows summarised beta connectivity analysis for all studies. Figure 7.2A shows a connectivity map of only those connections that are valid in *all* studies. There were 68 connections that were valid at a threshold of 50%. Figure 7.2B shows t-tests of only these valid connections for each study. Reduced connectivity in these connections in cases is evident in most studies although the effects appear weaker in Study 2, Study 4 and Study 7. Mixed (both increased and decreased) connectivity is seen in Study 4, 5 and 7. This is also

evident when reviewing boxplots of mean t-statistics in Figure 7.2C. For studies 2-7, the mean is close to zero and boxes cross zero for studies 4-7 suggesting minimal differences between cases and controls. Again, when looking at t-statistics for each connection and study, lines are fairly close to zero aside from Study 1 (both conditions).

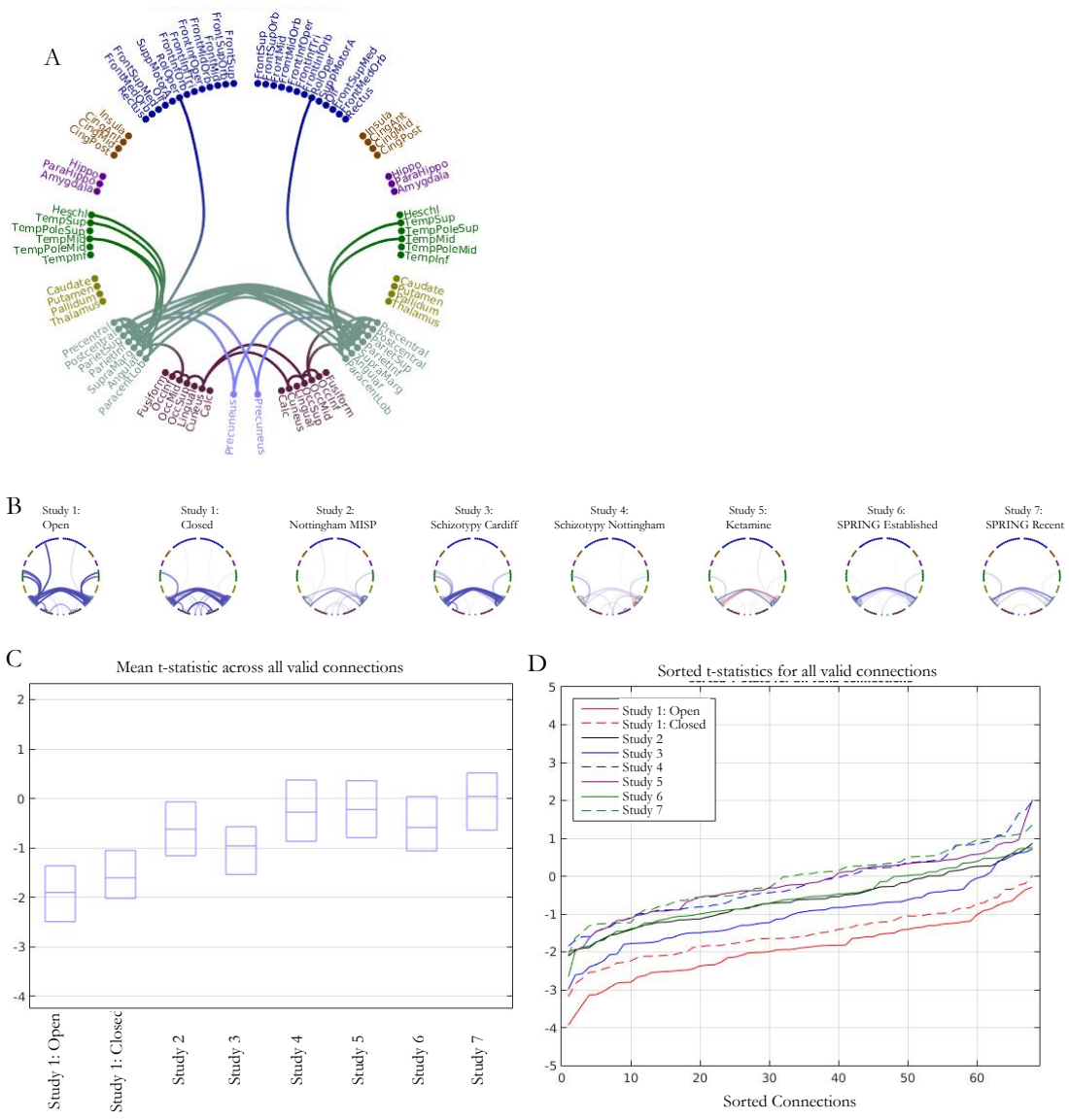


Figure 7.2 Summarized Beta Connectivity across all studies
A. Connectivity map showing only connections valid in all studies.
B. t-test of valid connections in each study. Blue=reduced connectivity in cases. Red=increased connectivity in cases.
C. Box plots of mean t-statistics across all valid connections for each study.
D. Graph of sorted t-statistics for valid connections for each study.

7.4.1 Meta-Analysis Results

Figure 7.3 shows a forest plot of Alpha connectivity including all studies. Measures of heterogeneity including Chi^2 (12.93, p=0.04), Tau^2 (a measure of between study variance, 0.13) and I^2 (percentage of variation across studies that is due to heterogeneity rather than chance, 54%) suggest statistically significant heterogeneity between studies. The test for overall effect when combining studies indicates a 1.86 standard deviation difference across studies but this is non-significant (Z=1.86, p=0.06).

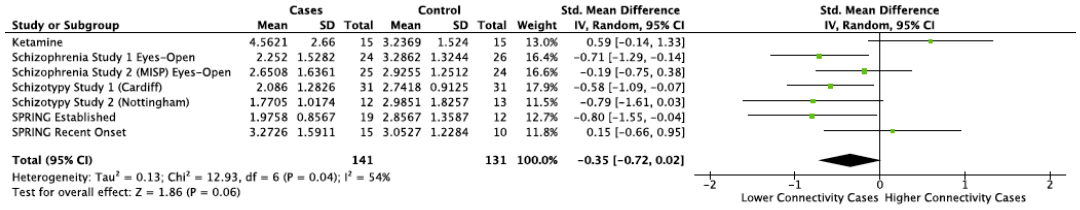


Figure 7.3 Alpha Connectivity Forest Plot Including All Studies

On reflection and further review of the literature, I repeated the analysis excluding the Ketamine study. This was because, firstly, connectivity following Ketamine administration was increased, suggesting a very different effect to that seen in schizophrenia. Secondly, most of the other studies were of participants with later stages of schizophrenia and as discussed, previous studies suggest that Ketamine does not model later stages of schizophrenia well. Therefore, it would make more sense to exclude it from grouped analysis.

A forest plot of Alpha connectivity for all studies except the Ketamine study is shown in Figure 7.4. When removing the Ketamine study, heterogeneity between studies reduced and was no longer significant (Tau^2=0.01; Chi^2=5.4, p=0.37; I^2=7%). The test for overall effect when combining studies was again statistically significant with now highly significant reductions in alpha connectivity in cases (Z=3.57, p=0.0004).

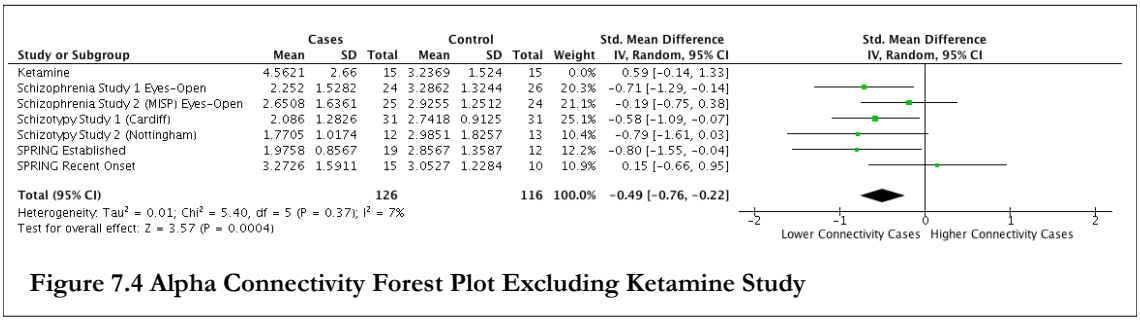


Figure 7.4 Alpha Connectivity Forest Plot Excluding Ketamine Study

A forest plot of Beta connectivity for all studies is shown in Figure 7.5. Heterogeneity between studies was minimal and non-significant (Tau^2=0; Chi^2=5.42, p=0.49; I^2=0%). The test for overall effect when combining studies was again statistically significant with significant reductions in beta connectivity in cases (Z=2.92, p=0.004).

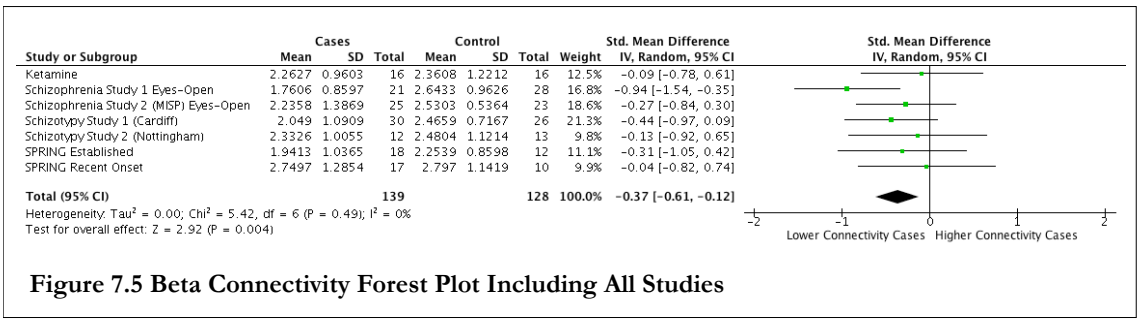


Figure 7.5 Beta Connectivity Forest Plot Including All Studies

A forest plot of Beta connectivity for all studies except the Ketamine study is shown in Figure 7.6. Heterogeneity between studies was minimal and non-significant (Tau^2=0; Chi^2=4.71, p=0.45; I^2=0%). The test for overall effect when combining studies was again statistically significant with significant reductions in beta connectivity in cases (Z=3.02, p=0.002).

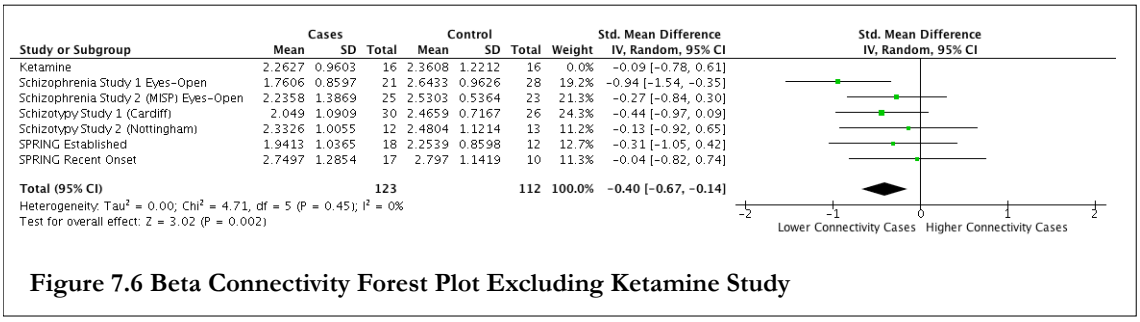


Figure 7.6 Beta Connectivity Forest Plot Excluding Ketamine Study

7.5 Discussion

Across seven studies of schizophrenia, the continuum of schizophrenia (schizotypy) and a model of schizophrenia (ketamine) I found somewhat converging results in MEG resting-state connectivity, particularly in the alpha frequency band. I found reduced connectivity in patients with schizophrenia in two studies where no stage or duration of illness was specified. I also found reduced connectivity in a group of patients with established (treatment resistant) schizophrenia. I did not find differences in connectivity in a group of patients with recent onset psychosis (within 12 weeks of treatment). These results alone suggest that there may be a difference in connectivity between the early stages and later stages of the disorder and may help to explain some of the heterogeneity in the literature (Bowyer et al., 2015, Kim et al., 2014, Hinkley et al., 2011). When considering these results in the context of our findings in a study of an NMDA receptor antagonist (ketamine), I found increased connectivity in healthy individuals following ketamine administration. This reflects other work finding increased connectivity following ketamine administration (Anticevic et al., 2015a, Driesen et al., 2013a, Hoflich et al., 2015, Rivolta et al., 2015). This therefore suggests that Ketamine does not model established psychosis well, (where we predominantly see reduced connectivity.)

Interestingly, I also found reduced connectivity in two groups of healthy participants with high schizotypy scores. This suggests that there may be biological continuity between subclinical psychotic traits in healthy individuals and clinically diagnosable schizophrenia. Given the lack of significant changes in connectivity in patients with recent onset psychosis, this suggests that the link between connectivity changes seen in schizotypy and those seen in schizophrenia is not simple. Study design may explain some of the differences I have found. Since the recent onset group were quite heterogeneous in themselves (with diagnoses of

schizophreniform disorder or schizophrenia), they may not all go on to develop established schizophrenia and therefore any changes in connectivity may be diluted by this heterogeneity.

Results may also be partially explained by the comparator groups used. For all of the schizophrenia studies, I compared patients with healthy controls. These healthy controls were not assessed for schizotypy and therefore not selected on this basis. However, for the schizotypy studies, cases with high schizotypy were compared with controls with low schizotypy. It may therefore stand that for the recent onset group, differences may have been apparent had we compared them to individuals with low schizotypy.

When combining results using a meta-analytic approach, I found reduced connectivity in cases (referring to patients with schizophrenia, healthy controls with high schizotypy and healthy controls following ketamine administration) in both the alpha and beta frequency bands.

Given that connectivity was actually increased in healthy controls following ketamine administration, this pooled result was stronger and more significant when removing the Ketamine study from the pooled analysis.

7.6 Conclusion

In summary, this work supports the dysconnectivity hypothesis of schizophrenia, specifically finding reduced posterior alpha band connectivity in patients with schizophrenia. Such changes are found predominantly in the later stages of the disorder suggesting possible progressive changes throughout its course. I found increased connectivity following ketamine administration in the same frequency band and region suggesting the drug does not model later stages of the disorder well (where we predominantly see hypo-connectivity).

Based upon previous research, it may be that ketamine models the earlier stages of schizophrenia better than later stages. Finally, I also found hypo-connectivity in healthy volunteers with high schizotypy scores suggesting some biological continuity between subclinical symptoms and diagnosable schizophrenia. When pooled together, I found significantly reduced connectivity in cases with schizophrenia and schizotypy.

Chapter 8 The Impact of Disease Stage Upon MRS GABA in Schizophrenia and Correlations with MEG Functional Connectivity

8.1 Rationale

GABA has been implicated in the pathogenesis of schizophrenia for many years due to multiple post-mortem studies finding deficits in the enzyme responsible for its production. However, results from in vivo studies using Magnetic Resonance Spectroscopy (MRS) have been inconsistent.

This study will investigate differences in occipital GABA between patients with differing stages of schizophrenia and healthy controls using MRS. I will also explore the association between functional connectivity and GABA in order to elucidate the link between dysconnectivity seen in schizophrenia and any neurochemical deficits.

8.2 Background

As discussed in Chapter 6, a substantial body of evidence points towards the GABA and glutamate hypotheses in the aetiology of schizophrenia. These posit that hypofunction of the NMDA receptor (an ionotropic glutamate receptor) results in reduced excitation of GABAergic interneurons which in turn results in the disinhibition of glutamatergic pyramidal neurones (Homayoun and Moghaddam, 2007). This results in a change in dopamine firing patterns and increased dopamine release (Jackson et al., 2004). In addition to causing psychosis through this resultant hyperdopaminergic state, excess glutamate may also result in excitotoxicity and neuronal death.

GABA was first implicated in schizophrenia after multiple post-mortem studies of patients with the disorder showed reduced GAD67 mRNA and protein levels (Curley et al., 2011, Thompson et al., 2009). GAD67 is the enzyme responsible for most cortical GABA production and GAD67 complete knockout mice show a 93% reduction in GABA and die within hours of birth (Asada et al., 1997). Other studies of mice with incomplete knockout of GAD67 show learning and social behaviour deficits (Zhang et al., 2014a) thus linking deficits in GABA synthesis with symptoms seen in schizophrenia. In other animal studies, GABAergic interneuron density is reduced following the administration of NMDA antagonists (Braun et al., 2007, Keilhoff et al., 2004, Zhou et al., 2015). This therefore links the NMDAR hypofunction hypothesis and the GABAergic findings seen in schizophrenia. However, although post mortem studies clearly point towards disruption in GABA, results from in vivo studies of GABA using MRS in patients with schizophrenia are inconsistent.

Such studies have explored varying brain regions and stages of schizophrenia. Several studies of the prefrontal cortex have found no difference (Tayoshi et al., 2010, Brandt et al., 2016) or reduced GABA in schizophrenia (Rowland et al., 2013, Marsman et al., 2014, Rowland et al., 2015). Studies of the occipital cortex have also found reduced GABA in schizophrenia (Yoon et al., 2010, Kelemen et al., 2013, Thakkar et al., 2017). One study of the hippocampus (Stan et al., 2015) and one of the dorsolateral prefrontal cortex (Chen et al., 2014) found no difference in GABA between patients with schizophrenia and controls. Other studies have found increased GABA in schizophrenia in the dorsal anterior cingulate cortex (Ongur et al., 2010) and the medial prefrontal cortex (Kegeles et al., 2012). A recent meta-analysis by (Egerton et al., 2017) pooled studies into those exploring the medial frontal cortex, parieto-occipital cortex and striatum. Whilst there were reductions in GABA in these regions in patients with schizophrenia, effect sizes were small and did not reach statistical significance.

However, the meta-analysis revealed significant heterogeneity between studies and also included studies of Ultra High Risk groups.

Heterogeneity of results seen in MRS studies of GABA in schizophrenia may be due to methodological differences but could also be due to the age of participants, disease stage or antipsychotic exposure. In line with the hypothesis of accelerated ageing in schizophrenia (Kirkpatrick et al., 2008), MRS studies of GABA in schizophrenia have found differences between younger and older patients. For example, Rowland et al. (2015) found reduced GABA in the medial frontal cortex in older patients with schizophrenia whereas no difference in younger patients with schizophrenia. They also found a more rapid age related decline in GABA in patients with schizophrenia than with controls. Such findings however, may represent differences in illness duration or medication exposure as some studies have found an inverse relationship between antipsychotic medication dose and MRS GABA (Tayoshi et al., 2010, Kegeles et al., 2012, Marenco et al., 2016). However, confounders such as illness severity may explain this relationship as other studies fail to find this association (Kelemen et al., 2013, Goto et al., 2010). In addition, animal studies have found no change (Bustillo et al., 2006, McLoughlin et al., 2009) or increases (Konopaske et al., 2013) in GABA following antipsychotic exposure.

GABA is important for the synchronised oscillatory activity of pyramidal neurones and deficits in the GABAergic system have been linked to cognitive deficits such as working memory impairment seen in schizophrenia (Lewis et al., 2005, Volk and Lewis, 2005). Studies have also found an association between inhibitory tone and more widespread functional connectivity (Stagg et al., 2014, Kapogiannis et al., 2013), thereby linking the dysconnectivity hypothesis (Stephan et al., 2009a) and hypotheses implicating the GABAergic system in schizophrenia (Gonzalez-Burgos et al., 2011).

8.3 Aims and Hypotheses

Given the significant heterogeneity of results seen in MRS studies of GABA in schizophrenia and evidence suggesting that disease stage may have an impact upon GABA levels (Marenco et al., 2016, Kegeles et al., 2012, Rowland et al., 2013, Rowland et al., 2015), I sought to explore GABA levels in different stages of schizophrenia. Since most studies have seen a reduction of GABA in schizophrenia, I expected to see lower GABA which was more pronounced in later stages of the disorder than early stages.

Previous work in this thesis has focussed upon functional connectivity and I therefore also sought to explore the relationship between GABA and functional connectivity in individuals with schizophrenia. To my knowledge, no other studies in schizophrenia have explored this association using MEG. However, studies of healthy individuals have found an inverse relationship between GABA and functional connectivity (using fMRI) (Stagg et al., 2014, Kapogiannis et al., 2013) and I therefore expected this relationship to be disrupted in schizophrenia.

8.4 Materials and Methods

8.4.1 Participants

This study has been previously outlined in Chapter 5. The study was ethically approved in line with national practices. Participants gave written, informed consent prior to taking part.

The data used in this study were acquired as part of a multi-centre study of schizophrenia entitled SPRING (The Study of Psychosis and the Role of Inflammation, GABA and Glutamate). The study was conducted at Cardiff University, The University of Nottingham and The University of Manchester. In this chapter, I will only present data collected at Cardiff

University by myself and Dr Loes Koelewijn. There were two arms of the study, one investigating recent onset psychosis and the other investigating established psychosis.

8.4.1.1 Recent Onset Psychosis Group

14 participants within 5 years of a DSM-IV diagnosis of schizophrenia or schizophreniform disorder (10 males, 4 females; mean age: 24 +/-4.36, age range: 18-31) took part in the study. Participants had no exposure (n=1) or minimal exposure (<12 weeks) (n=13) to antipsychotic medication.

8.4.1.2 Established Psychosis Group

20 participants with more than 10 years' history of a DSM-IV diagnosis of schizophrenia (17 males, 3 females; mean age: 39 +/-7.78, age range: 27-54) took part in the study. Participants had at least eight weeks' stable treatment prior to taking part in the study.

Cases were recruited through local Community Mental Health Teams (CMHTs), specialist Early Intervention for Psychosis Services and Clozapine clinics. Participants were diagnosed through clinical assessment and case note review followed by verification by clinical consensus.

8.4.1.3 Healthy Control Groups

Both case groups had an age, sex and parental occupation matched group of 10 healthy control participants. These were recruited locally through an online advert and the University noticeboard. The recent onset control group consisted of 7 males, 3 females; mean age: 23 +/-3.03, age range: 18-26. The established control group consisted of 10 males, 2 females; mean age: 39 +/-7.78, age range: 29-54.

8.4.1.4 Inclusion and Exclusion Criteria

For all groups inclusion criteria were; male or female, aged 18 - 55 years, ability to understand and willing to give written informed consent and English as first language or fluent. Exclusion criteria for case groups were; clinically significant neurological disorder, history of head injury with loss of consciousness >5 minutes, current harmful use of, or recent dependence on, psychoactive substances (excluding nicotine), contraindications for MR scanning (e.g. claustrophobia, pregnancy etc). Exclusion criteria for control groups were; personal history of psychosis or related disorder as determined by MINI (-international neuropsychiatric interview), current or recent (within 2 years) presence of depressive symptoms or treatment with antidepressant medication, current use of any medication which may interfere with the study, first degree relative with a history of psychosis, clinically significant neurological disorder, history of head injury with loss of consciousness >5 minutes.

8.4.2 MRI Data Acquisition and Analysis

Individual anatomical MRIs (1-mm isotropic, T1-weighted FSPGR) were acquired using a 3.0 T MRI scanner (General Electric).

GABA was quantified in the occipital lobe (OCC). The occipital voxel was 3cm by 3cm by 3cm and positioned as far as possible parallel with the junction between the occipital lobe and the cerebellum. (See Figure 8.1.)

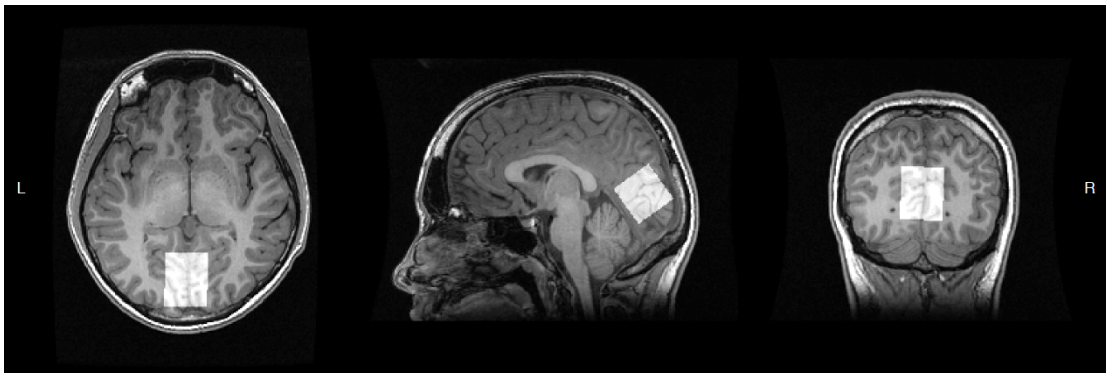


Figure 8.1 Occipital Voxel Position

GABA was detected with a MEGA-PRESS acquisition. In these acquisitions (TE/TR=68/2000ms), Gaussian editing pulses (16ms duration) were placed at either 1.9ppm (ON) or 7.5ppm (OFF), to give a GABA edited spectra.

GABA was then quantified using Gannet 2.0 toolkit (http://www.gabamrs.com). Following phase correction of individual spectra, "ON" and "OFF" were subtracted resulting in the edited spectrum. The edited GABA peak was then modelled as a single Gaussian, enabling quantification of GABA as the integral of the area under the peak. GABA concentration was then estimated relative to both water and, separately creatine. Fit error was calculated by dividing the standard deviation of the fitting residual by the amplitude of the fitted peak.

Again, using Gannet, the voxel was segmented into grey matter, white matter and CSF and for GABA/H2O measurements, concentrations were then corrected for the proportions of these (Gasparovic et al., 2006).

Spectra were excluded if the fit error was above 10% or there was a poor fit on visual inspection. An example of a poorly fit GABA spectrum can be seen in Figure 8.2. The blue line represents the actual spectra and the red line represents the model fit. In this example, the fit is poor and therefore unlikely to give a good estimation of GABA. Figure 8.3 shows an example of a well fit GABA spectra. Finally, outliers were excluded using a procedure in

which only GABA measures within plus or minus two standard deviations of the mean, within each sub-cohort, were included in further analysis. A t-test was then performed in order to assess between group differences.

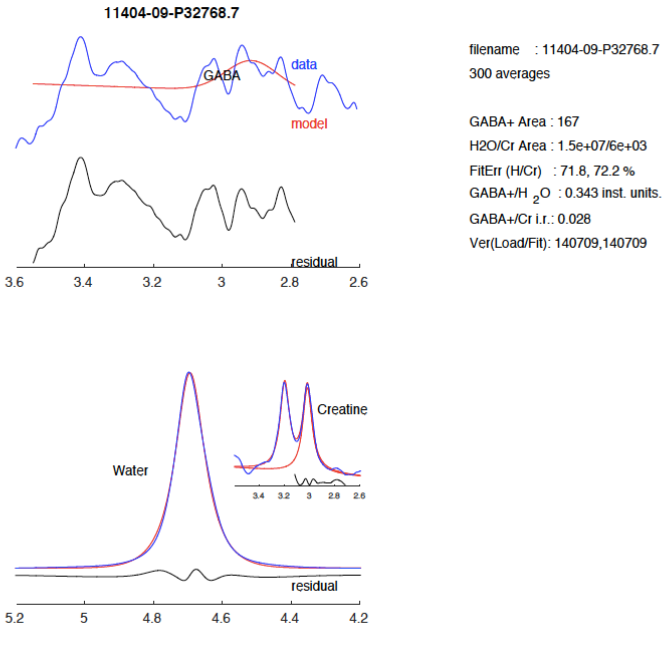


Figure 8.2 GannetFit Output example of poorly fit GABA spectre

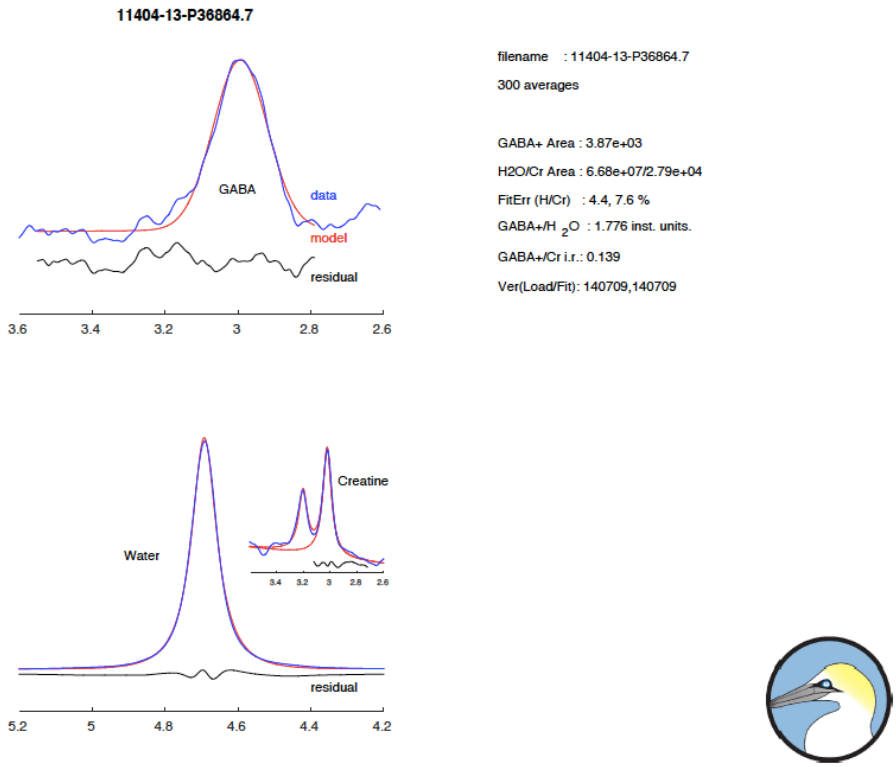


Figure 8.3 GannetFit Output example of well fit GABA spectre

8.4.3 Correlations with MEG-Derived Functional Connectivity

Using results from the connectivity analysis outlined in previous chapters, we analysed correlations between GABA/H2O and GABA/Cr and connectivity. For this analysis, we used only connections that were valid in all studies. We corrected for multiple comparisons using randomisation testing (5000 iterations).

All SPRING controls (i.e. those matched to both the recent onset and established groups) were used as a control group for comparison.

8.5 Results

8.5.1 GABA Analysis for the Recent Onset Psychosis Group

Mean and standard error for GABA/Cr and GABA/H2O is shown in Figure 8.3. Outlier rejection led to the removal of one recent onset participant for GABA/H2O and one for GABA/Cr.

There were no significant differences in GABA/Cr ($t(18)=-1.13$, $p=0.28$), GABA/H2O ($t(19)=-0.99$, $p=0.33$) between recent onset participants and controls.

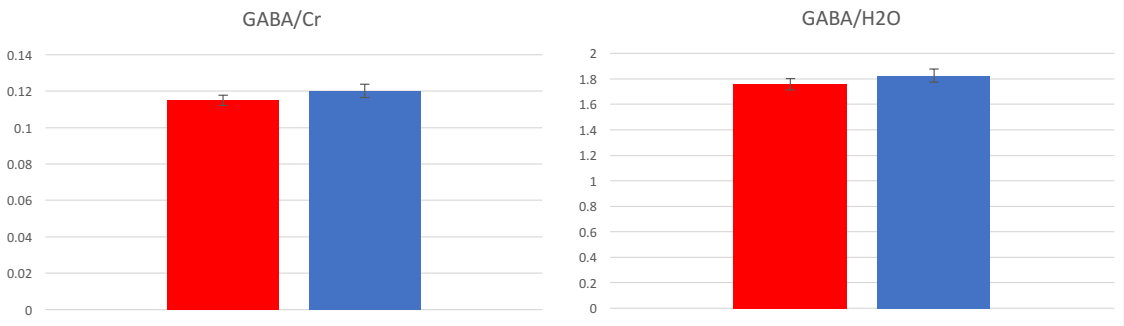


Figure 8.3 Recent Onset patients vs Controls GABA Analysis
Bars represent mean occipital GABA/Cr and GABA/H2O.
Error bars represent +/-1 Standard Error of the mean.
Red=Recent Onset Patients, Blue=Controls

Figure 8.4 shows a scatter plot of GABA/H2O and GABA/Cr for recent onset participants and controls.

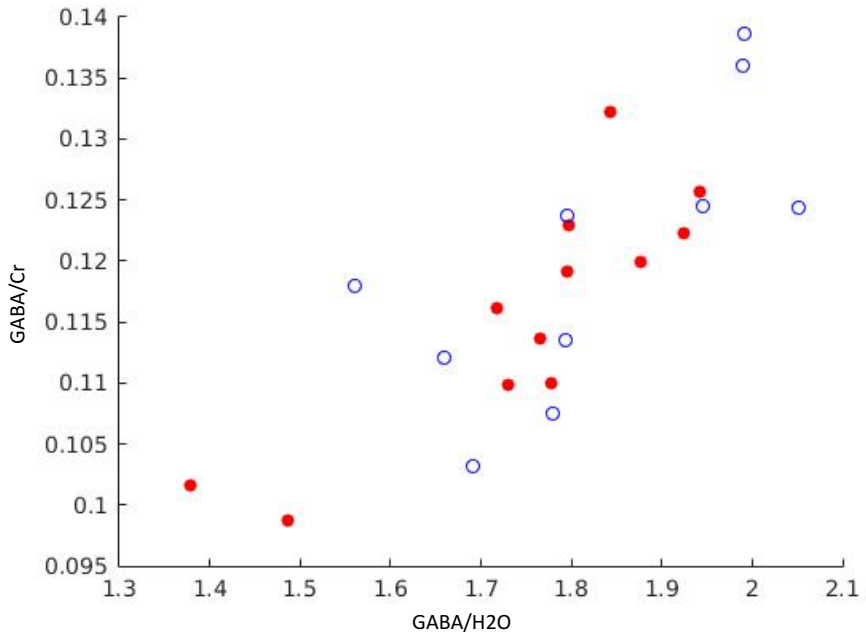


Figure 8.4 Recent Onset GABA/H2O vs GABA/Cr
Red=Recent Onset Patients, Blue Circles=Controls

8.5.2 GABA Analysis for the Established Schizophrenia Group

Mean and standard error for GABA/Cr and GABA/H2O is shown in Figure 8.5. Two established participants were excluded due to the fit error being greater than 10%. Outlier rejection led to the removal of one established participant (for both GABA/Cr and GABA/H2O) and one established control for GABA/H2O and one for GABA/Cr.

There was a trend towards lower GABA/Cr ($t(24)=-1.79$, $p=0.086$) in established participants compared to controls but this did not meet statistical significance.

GABA/H2O (t(26)=-2.37, p=0.025) was significantly lower in established participants than controls.

This trend can be seen in Figure 8.6 where established participants (red dots) are positioned towards the lower left of the graph whereas controls (blue circles) are more towards the top right.

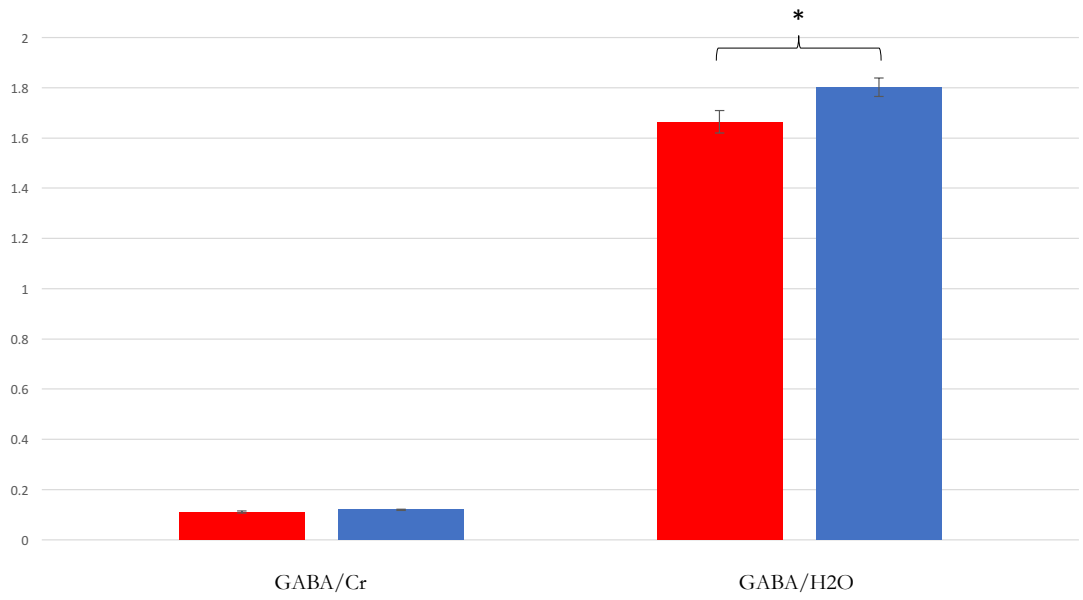


Figure 8.5 Established patients vs Controls GABA Analysis
Bars represent mean occipital GABA/Cr and GABA/H2O.
Error bars represent +/-1 Standard Error of the mean.
Red=Established Patients, Blue=Controls
* = t-test between groups, p<0.05

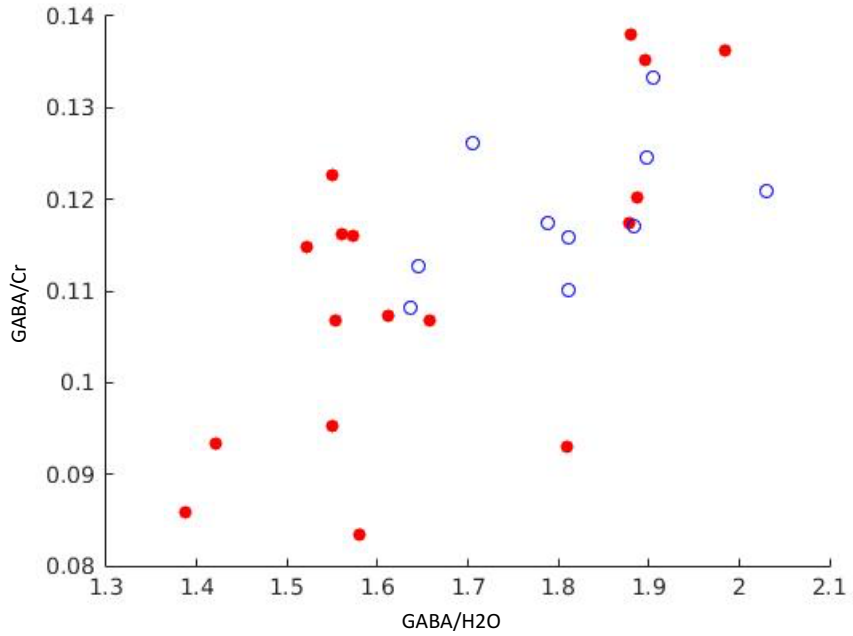


Figure 8.6 Established GABA/H2O vs GABA/Cr
Red=Established Patients, Blue Circles=Controls

8.5.3 Correlations with Connectivity

8.5.3.1 Correlations between GABA and Alpha Connectivity

Figure 8.7 shows GABA/H2O and GABA/Cr correlations with connectivity for SPRING Controls, SPRING Recent Onset Participants and SPRING Established Participants. For the SPRING Controls, there is widespread positive correlation between GABA and connectivity. For SPRING Recent Onset Participants, there are positive and negative correlations between GABA and connectivity in the occipital, parietal and temporal regions. These correlations are more localised to the parieto-occipital cortex in SPRING established participants and are mostly negative, suggesting higher connectivity with lower GABA levels. However, none of these findings reached statistical significance after randomisation testing.

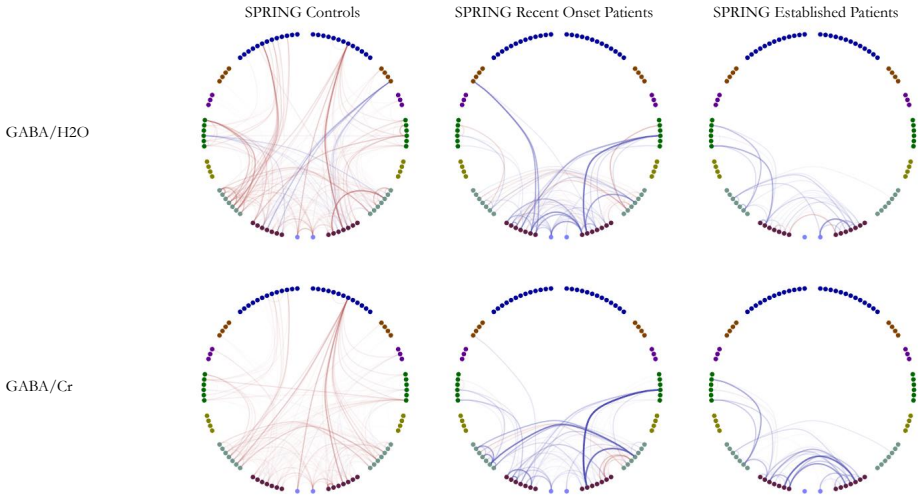


Figure 8.7 Uncorrected Correlations of GABA and Alpha Connectivity
Connectivity maps showing correlations of GABA with connections valid in all studies.
Red lines=positive correlation between GABA and connectivity
Blue lines=negative correlation between GABA and connectivity

Figure 8.8 shows a bar-graph of all of these correlation coefficients, for all studies, with blue bars representing control groups and red bars representing cases. The horizontal line represents a correlation coefficient of zero, with bars above this representing positive correlations between GABA and connectivity and bars below this representing negative correlations. Again, the control group shows a mixed picture but with mostly positive correlations between GABA and connectivity whereas case groups have more mixed correlations between GABA and connectivity.

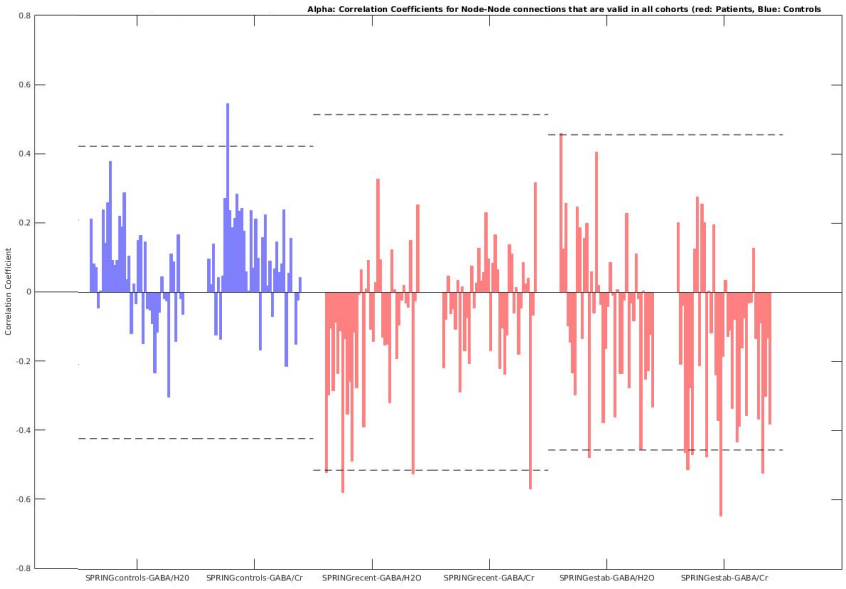


Figure 8.8 Graphs of Correlation Coefficients for GABA and Alpha Connectivity
Blue=Control Groups
Red=Patient Groups
Dotted lines=$p<0.05$ significance

Figure 8.9 compares correlations seen with GABA/H2O and those seen with GABA/Cr., Data points are fairly widespread, with case groups showing a mixed picture of correlation between GABA and connectivity. On the whole, for all groups, there appears to be a positive correlation between alpha connectivity and GABA/H2O or GABA/Cr. This suggests that most of the inter-subject variance in these correlations is driven by variance in GABA rather than creatine or water.

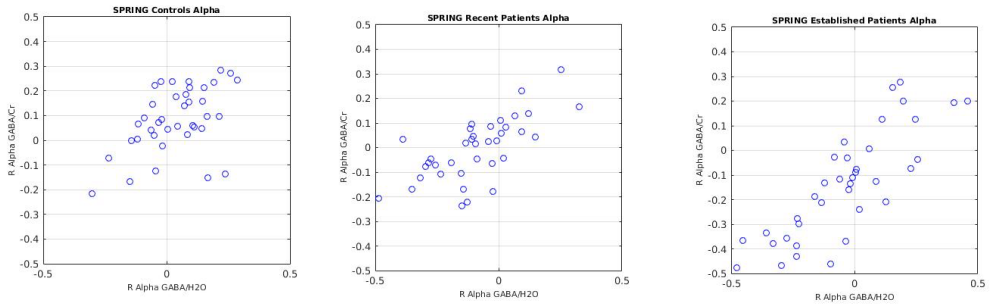


Figure 8.9 Correlation between R values for GABA/H2O and GABA Cr
Horizontal axes=GABA/H2O R values, Vertical axes=GABA/Cr R values,
Blue circles=R values
From left to right: SPRING Controls, SPRING Recent Onset Patients, SPRING Established Patients

8.5.3.2 Correlations between GABA and Beta Connectivity

Figure 8.10 shows GABA/H2O and GABA/Cr correlations with beta connectivity for SPRING Controls, SPRING Recent Onset Participants and SPRING Established Participants. For the SPRING Controls, there is widespread positive correlation between GABA and beta connectivity. For SPRING Recent Onset Participants, this pattern is more mixed but more closely resembles the control group. SPRING Established Participants show a very different pattern, with negative correlations between GABA/H2O and GABA/Cr and connectivity in predominantly parieto-occipital regions. Again, after randomisation testing, none of these correlations reach statistical significance.

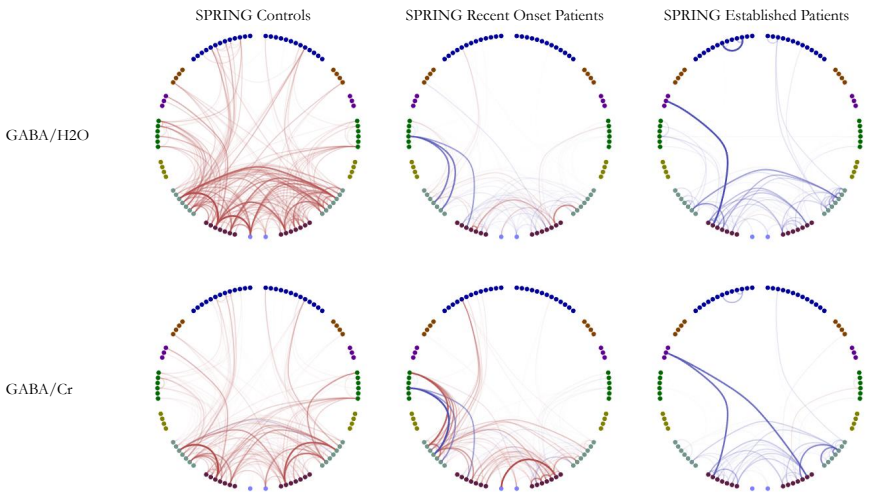


Figure 8.10 Uncorrected Correlations of GABA and Beta Connectivity
Connectivity maps showing correlations of GABA with connections valid in all studies.
Red lines=positive correlation between GABA and connectivity
Blue lines=negative correlation between GABA and connectivity

A graph of correlation coefficients in Figure 8.11 again shows a pattern of positive correlations between GABA (both GABA/H2O and GABA/Cr) in the control group, a mixed picture in the SPRING Recent Onset Participants and predominantly negative correlations between GABA and connectivity in the SPRING Established Participants. This is also reflected in Figure 8.12. In general, effects appear much stronger for correlations with beta connectivity than for those with alpha connectivity.

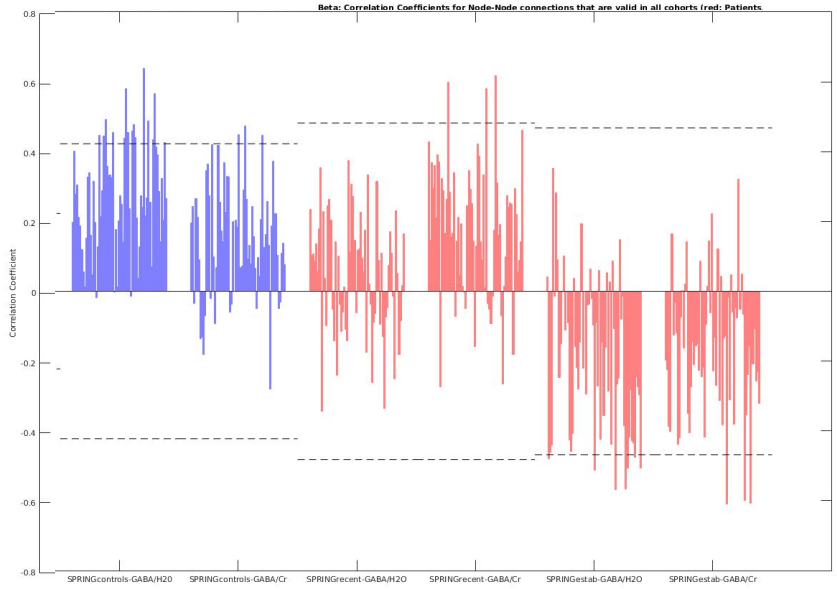


Figure 8.11 Graphs of Correlation Coefficients for GABA and Beta Connectivity
Blue=Control Groups
Red=Patient Groups
Dotted lines=$p<0.05$ significance

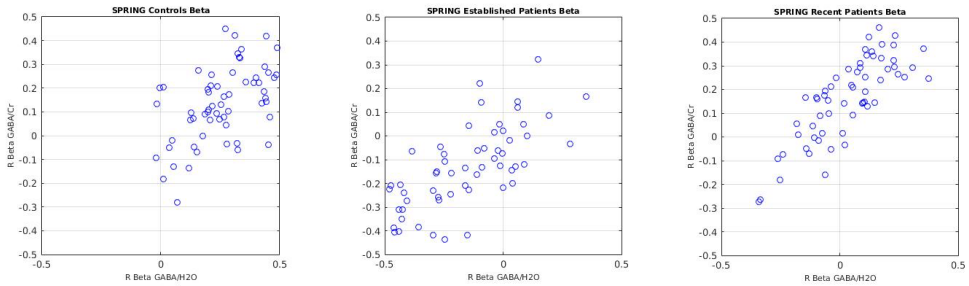


Figure 8.12 Correlation between R values for GABA/H2O and GABA Cr Correlations with Beta Connectivity
Horizontal axes=GABA/H2O R values, Vertical axes=GABA/Cr R values,
Blue circles=R values
From left to right: SPRING Controls, SPRING Recent Onset Patients, SPRING Established Patients

8.6 Discussion

Reduced GABA/H2O and a trend to reduced GABA/Cr was found in participants with established schizophrenia compared with controls. There was no significant difference in GABA between recent onset participants and controls. This replicates other studies finding reduced GABA in the occipital cortex in patients with schizophrenia (Yoon et al., 2010, Kelemen et al., 2013, Thakkar et al., 2017). However, in contrast to our findings of reduced GABA only in established participants and not in recent onset participants, (Kelemen et al., 2013) found reductions in GABA in drug naïve first episode patients. In addition, Yoon et al. (2010) found a 10% reduction in occipital GABA in a mixed group of patients with both chronic and recent onset schizophrenia.

Our findings of reduced GABA in older individuals with established schizophrenia but no difference in GABA in recent onset (younger) patients with schizophrenia reflect findings from Rowland et al. (2015). These results may add support to the accelerated ageing hypothesis of schizophrenia and white matter studies finding more rapid decline in patients with age (Kochunov et al., 2014, Wright et al., 2014). Although, this is with the caveat that our study is cross sectional and to fully understand longitudinal changes in the disorder would require a longitudinal study such as the study of structural changes in schizophrenia by Schnack et al. (2016).

This study found reductions in GABA/H2O but only a trend to a reduction in GABA/Cr in established participants with schizophrenia. Interestingly, Marenco et al. (2016) found reduced GABA/Cr in medicated patients but no difference in GABA/H2O. This suggests that the choice of reference to GABA is important especially since some studies have found differences in creatine between patients with schizophrenia and controls (Theberge et al., 2007, Ongur et al., 2009, Meyer et al., 2016). Our different strength of findings in

GABA/H2O and GABA/Cr may therefore be due to a difference in creatine in participants with schizophrenia.

For the second part of this study, I explored associations between connectivity (measured using amplitude envelope correlations in MEG) and GABA. The strongest effects were found between beta connectivity and GABA. The control group showed a consistent positive correlation between connectivity and both GABA/H2O and GABA/Cr. Effects for correlations in the Recent Onset Patient group showed an intermediate pattern with both positive and negative correlations. Established patients showed a much different pattern of correlation between GABA and connectivity with much more negative correlations, predominantly in the parieto-occipital cortex. Whilst these correlations did not reach statistical significance, the differing pattern between recent onset patients and established patients is interesting and again may provide support for progressive changes throughout the course of the disorder.

Studies of healthy individuals have found correlations between functional connectivity measured with fMRI and GABA. For example, Stagg et al. (2014) found an inverse correlation between GABA and resting-state motor network connectivity. They also found that decreasing GABA with anodal tDCS resulted in increased functional connectivity. Kapogiannis et al. (2013) also found a negative correlation between Default Mode Network (DMN) GABA (and glutamate) and DMN functional connectivity using fMRI. This correlation was only evident in the DMN suggesting that there is a relationship between regional (but not global) functional connectivity and local inhibitory tone. Similarly, Shukla et al. (2018) found a significant negative correlation between GABA and fMRI functional connectivity in the ACC and no correlation in patients with schizophrenia. This, along with our results, suggests that GABA is important in the modulation of functional connectivity and that the relationship between brain chemistry and functional connectivity is perturbed

in schizophrenia. However, our study using MEG and MRS suggests that in healthy controls GABA is positively correlated with functional connectivity and that this relationship reverses in schizophrenia. These somewhat differing results may be due to the different brain regions studied and the differing cohorts of patients included in the studies.

Multiple studies in both animals and humans have explored the relationship between GABA and beta oscillations. Task based studies using GABA agonists have shown increased amplitude of baseline beta power (Hall et al., 2010) and increased movement related beta desynchronization (MRBD) (Hall et al., 2011) in healthy controls. A study by Muthukumaraswamy et al. (2013), found increased amplitude of baseline beta power and enhanced MRBD following the administration of Tagabine, a GABA reuptake inhibitor. Taken together, these studies suggest that increased GABA may lead to increased beta and therefore increased connectivity. This would fit with our findings in healthy controls.

8.6.1 Limitations

Firstly, GABA as measured by MRS is a measure of total GABA in a specific voxel and represents GABA in presynaptic vesicles, cytoplasm and extracellular fluid. Studies suggest that these measurements may represent the inhibitory tone of a brain region rather than GABAergic synaptic activity (Stagg et al., 2011, Dyke et al., 2017).

As previously discussed, in this study, I have looked at patients in two stages of disease state and it is difficult to make longitudinal conclusions based upon cross-sectional data as confounders such as medication exposure and illness severity could impact upon results. In addition, sample sizes for the SPRING study were relatively small leading to weak statistical power.

8.7 Conclusion

In conclusion, I found reduced GABA/H2O in a group of participants with Established Schizophrenia. No differences in GABA were found in participants with Recent Onset psychosis. I also found differing patterns of correlation between participants with Established Schizophrenia and those with Recent Onset psychosis and controls. This may potentially support hypotheses of progressive changes occurring throughout the disorder. However, further longitudinal studies would elucidate this further.

Chapter 9 General Discussion

This thesis utilised amplitude-amplitude coupling as a measure of functional connectivity in MEG in order to explore the dysconnectivity hypothesis of schizophrenia. In addition, functional connectivity was used to link dysconnectivity in schizophrenia with the continuum hypothesis of schizophrenia and ketamine, a model of schizophrenia. I also explored the neurochemistry of schizophrenia (specifically GABA) using MRS and its link with dysconnectivity.

9.1 Summary of Findings

Chapter 3 explored functional connectivity in two groups of participants with schizophrenia. Using amplitude-amplitude coupling in MEG, I found reduced functional connectivity in one cohort of participants with schizophrenia in the alpha and beta frequency bands. In a second study, I also found a negative correlation between beta band connectivity and illness severity suggesting connectivity in this frequency band was lower in more unwell cases. Whilst I was able to identify significant patient differences using MEG, I was not able to identify significant differences using fMRI. However, I found a positive correlation between t-tests of fMRI connectivity between cases and controls and beta band connectivity in MEG between cases and controls suggesting a link between the two measures.

Again, using amplitude-amplitude coupling in MEG, in Chapter 4, I explored the continuum hypothesis of schizophrenia by looking at functional connectivity in healthy individuals with high and low levels of schizotypy. Using this approach, I found reduced connectivity in two schizotypy studies in the alpha frequency band. Connectivity was globally reduced in two

studies in the groups with high schizotypy. In addition, connectivity of specific connections was reduced and these included parts of the Default Mode Network. In the beta frequency band, I found a significant correlation between t-test scores of differences between cases and controls in Schizophrenia Study 1 and t-test scores of differences between high and low schizotypy participants in Schizotypy Study 1.

In Chapter 5, I attempted to elucidate dysconnectivity in schizophrenia further by exploring functional connectivity in different stages of the disorder. Using the same methods as used in previous chapters, I found reduced connectivity in participants with (established) more than 10 years' history of schizophrenia in the alpha frequency band but not those within the early stages of psychosis.

Given my previously reported findings of dysconnectivity in schizophrenia, in Chapter 6, I explored the effects of ketamine (an NMDA antagonist and model of schizophrenia) upon connectivity in a healthy group of young men. The aim was to add evidence to the ketamine model of schizophrenia and further develop links between the glutamate hypothesis and the dysconnectivity hypothesis. Again, using the same method used in previous chapters, I found increased connectivity following acute ketamine administration in the alpha frequency band.

In Chapter 7, I summarised the findings in the previous four chapters using several approaches including a meta-analytic approach. When combining results using a meta-analytic approach, I found significantly reduced connectivity in cases (referring to

participants with schizophrenia, healthy controls with high schizotypy and healthy controls following ketamine administration) in both the alpha and beta frequency bands. Given that connectivity was actually increased in healthy controls following ketamine administration, this pooled result was stronger and more significant when removing the ketamine study from the pooled analysis.

Given neurochemical hypotheses of schizophrenia and heterogeneity within the MRS literature, the final chapter of this thesis sought to investigate differences in occipital GABA between participants with differing stages of schizophrenia and healthy controls using MRS. I also explored the association between functional connectivity and GABA in order to elucidate the link between dysconnectivity seen in schizophrenia and any neurochemical deficits. I found lower GABA/H2O and a trend to reduced GABA/Cr in participants with established schizophrenia compared with controls but did not find a difference in GABA between recent onset participants and controls. In addition, there was a positive correlation between GABA and beta connectivity in healthy individuals that was not found in cases with schizophrenia.

9.2 Implications of main findings and future work

9.2.1 Reduced functional connectivity in established schizophrenia:

Resting-state dysconnectivity has been repeatedly found in patients with schizophrenia. However, few studies have used MEG to probe resting-state connectivity in schizophrenia and of those that have, results are heterogeneous (Bowyer et al., 2015, Hinkley et al., 2011, Kim et al., 2014). Through utilising amplitude-amplitude coupling in MEG, I found reduced resting-state functional connectivity in the alpha and beta frequency bands in three separate

samples of participants with schizophrenia when restricting analysis to connections that were valid in all studies. In addition, in one study, there was reduced alpha and beta connectivity in participants with schizophrenia in two different conditions; eyes-open and eyes-closed. These results are consistent with research finding dysconnectivity in schizophrenia, particularly those finding reduced parietal and occipital connectivity in patients with schizophrenia (Henseler et al., 2010, Zhuo et al., 2014, Wende et al., 2015). In addition, a recent study by Hirvonen et al. (2017) also found reduced synchronisation in the visual cortex in patients with schizophrenia, however, this was during a perceptual task within the 30-120Hz frequency range.

Alpha oscillations are most prominently seen in the parietal occipital cortex at rest with the eyes-closed (Scheeringa et al., 2012) and alpha power is considered to reflect top-down inhibitory control processes (Klimesch et al., 2007). Increased posterior alpha power results in reduced connectivity with other brain regions. One study suggests that enhanced alpha power during tasks requiring internal attention may inhibit visual activity, consequently preventing disruption by external sensory information (Mo et al., 2013). Whilst alpha power was not explored in this study, power fluctuations may have therefore had an impact upon connectivity measures.

I did not find reduced connectivity in participants with recent onset psychosis, suggesting a difference between these individuals and those with more established disorder. This is consistent with other studies of functional connectivity finding differences between the early and later stages of the disorder (Anticevic et al., 2015a) as well as those looking at changes in structural connectivity throughout the course of the disorder (Friedman et al., 2008, Kong et al., 2011).

Given the heterogeneity in the literature, it may therefore be wise in future studies to consistently divide patients according to disease stage. Alternatively, a longitudinal design may also be useful to explore the impact of duration of illness further.

Other measures of connectivity such as phase-phase and phase-amplitude correlations could also be used. Also, given the excellent temporal resolution of MEG, which is not really exploited using the current methodology, dynamic connectivity measures could be used (O'Neill et al., 2017).

9.2.2 Reduced functional connectivity in schizotypy:

This finding lends further support to the hypothesis that there is neurobiological continuity between sub-clinical psychotic symptoms and clinically diagnosable schizophrenia. The finding of reduced functional connectivity in the alpha frequency band in two separate cohorts of individuals with high schizotypy mirrors our findings in several cohorts of patients with schizophrenia. This is perhaps surprising since, if we hypothesise that schizotypy represents a less severe form of clinically diagnosable psychosis, we would expect there to be a progressive deterioration in functional connectivity between schizotypy, the early stages of psychosis and later stages of psychosis. However, I did not find changes in functional connectivity in participants with recent onset psychosis and the findings in schizotypy are more similar to those of patients with established schizophrenia. It may be that whilst connectivity changes in schizotypy are similar to those seen in established schizophrenia, such individuals possess protective factors that prevent transition into diagnosable schizophrenia despite dysconnectivity. Results may also be partially explained by comparator groups used in the studies. For all of the schizophrenia studies, I compared cases with healthy controls. These healthy controls were not assessed for schizotypy and therefore not selected

on this basis. However, for the schizotypy studies, cases with high schizotypy were compared with controls with low schizotypy. It may therefore stand that for the recent onset group, differences may have been apparent had we compared them to individuals with low schizotypy.

9.2.3 Increased functional connectivity following ketamine administration in healthy controls:

Such results are consistent with previous findings of increased connectivity following ketamine administration (Hoflich et al., 2015, Rivolta et al., 2015, Driesen et al., 2013a, Anticevic et al., 2015a). The previous finding of hypo-connectivity in schizophrenia, and particularly the later stages of schizophrenia, fits with the hypothesis that acute ketamine administration is a better model for earlier stages of schizophrenia rather than later stages of schizophrenia. Future studies could use this method to explore the impact of chronic ketamine use upon functional connectivity in order to elucidate this relationship further since other studies have found hypo-connectivity following chronic use (Liao et al., 2016).

9.2.4 Reduced occipital GABA in established schizophrenia but not in recent onset psychosis:

There is heterogeneity in MRS GABA findings in schizophrenia with some studies finding increased GABA, some finding reduced GABA and others finding no difference in patients with schizophrenia. Given my previous findings of differential stage related changes in functional connectivity, it is perhaps not surprising that I found changes in GABA *only* in established schizophrenia and not in recent onset psychosis. These results reflect findings from Rowland et al. (2015) and may add support to the accelerated ageing hypothesis of

schizophrenia and white matter studies finding more rapid decline in patients with age (Kochunov et al., 2014, Wright et al., 2014). Although, this is with the caveat that our study is cross-sectional and to fully understand longitudinal changes in the disorder would require a longitudinal study such as the study of structural changes in schizophrenia by Schnack et al. (2016). Again, it may be useful when designing future studies to take disease stage into consideration.

9.3 Methodological Considerations

In the studies exploring disease stage, I have found differences between the early and later stages of schizophrenia. Whilst this is consistent with research that suggests a progressive decline in schizophrenia, the cross-sectional design of the studies makes it difficult to make longitudinal conclusions.

We must consider the impact, for example, of medication exposure, upon any outcome measures since recent onset participants were only minimally exposed to antipsychotics whilst established participants received medication for a much longer duration. In the studies presented here, I did not find any correlation between connectivity and medication exposure suggesting that this has not had a large impact on results. However, in future, it may be useful to develop a longitudinal study exploring functional connectivity at in the same patients following different lengths of exposure to antipsychotics. Such studies however, are costly and vulnerable to attrition.

In addition to medication as a confounding factor, we must also consider the validity of comparing results from a study of individuals with recent onset psychosis and a study of those with established schizophrenia. Such groups may be inherently different since we know that prognosis in first episode psychosis is heterogeneous (Menezes et al., 2006). i.e. only a

proportion of those with recent onset psychosis will go on to have established schizophrenia. Again, a longitudinal study would elucidate this further.

Results from this thesis and in general within the neuroimaging of psychosis field reveal significant inconsistencies in results. This lack of consistency may be partially explained by low sample sizes and lack of joint analysis across groups. In particular, sample sizes were small in the control groups in Chapter 5. This limitation can be overcome to a degree through meta-analysis, (as performed in Chapter 7) in order to reach firmer conclusions.

9.4 Conclusions

This work supports the dysconnectivity hypothesis of schizophrenia, specifically finding reduced posterior alpha band connectivity in participants with schizophrenia. Such changes are found predominantly in the later stages of the disorder suggesting some progressive changes throughout its course. I found increased connectivity following ketamine administration in the same frequency band and region suggesting the drug does not model later stages of the disorder well (where we predominantly see hypo-connectivity). Given previous research, it may be that ketamine models the earlier stages of schizophrenia better than later stages. In addition, I found reduced GABA in later stages of schizophrenia but not in early stages, again suggesting progressive changes throughout the course of the disorder.

Finally, I also found hypo-connectivity in healthy volunteers with high schizotypy scores suggesting some biological continuity between subclinical symptoms and diagnosable schizophrenia.

Overall, these results add support to the dysconnectivity hypothesis, the GABA/glutamate hypothesis and the continuum hypothesis of schizophrenia.

9.5 Future Directions

Our differing findings in two groups of participants with "generic" schizophrenia (Chapter 3) as well as differing findings in recent onset psychosis and established schizophrenia (Chapter 5) highlight the challenges of studying such a heterogeneous condition. This is also reflected in previous research exploring structural connectivity, functional connectivity and neurochemistry in schizophrenia, whereby there isn't a clear, consistent picture across all studies. Case-control studies looking at different disease stages help to a certain degree but do not elucidate fully due to limitations previously discussed. Longitudinal studies may help further but these are costly and prone to issues such as attrition.

Overall, whilst research in schizophrenia has developed significantly over recent years, our understanding of the condition remains fairly limited and we continue to study schizophrenia as one condition despite it being evident that there is significant heterogeneity in the disorder. For example, it has been suggested that treatment resistant schizophrenia represents a different subgroup of schizophrenia. Also, in clinical practice, there are different diagnostic categories of the disorder.

One field of schizophrenia research that has seen huge progress over recent years is within genetics. The disorder has a heritability of around 80% suggesting that a large proportion of susceptibility is inherited (Hilker et al.). Through the pooling of extremely large cohorts of patients into Genome Wide Association Studies, numerous common alleles of small effect have been found to be implicated in schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics et al., 2014). In addition to such common polymorphisms, rare variants such as Single Nucleotide Variants (SNVs) and Copy Number Variations (CNVs) contribute to schizophrenia susceptibility with varying effect sizes across a large number of

genes (Rees et al., 2014). In addition, a proportion of genetic risk for schizophrenia is not inherited but occurs de novo (Kirov et al., 2012).

Therefore, these advancements in our understanding of the genetics of schizophrenia may aid us in understanding the neurobiology of the disorder further. For example, through the stratification of patients with schizophrenia according to genetic variants they possess. This leads on to a current study, Genetic Variants in Psychosis (GVIP), funded by the Wellcome Trust and the Brain and Behaviour Research Foundation that I am currently carrying out.

Pathogenic CNVs in schizophrenia have been found to converge upon genes involved in GABAergic and glutamatergic neurotransmission (Pocklington et al. (2014). In this thesis, the GABA/glutamate and dysconnectivity hypothesis of schizophrenia have been central and therefore the exploration of the impact of such rare variants upon neurobiology in schizophrenia seemed appropriate. Through the GVIP study, I am testing the hypothesis that participants with schizophrenia and CNVs/rare variants hitting GABA and glutamate pathways will display altered neural synchronization and neurochemistry, as measured by MEG and MRS respectively, compared to participants with schizophrenia but without significant CNVs/rare variants. This may allow development of current hypotheses of schizophrenia and improve our understanding of the heterogeneity of the disorder.

In addition, within the genetics field, very large multicentre studies have resulted in huge breakthroughs in our understanding of the genetics of schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics, 2014). This approach could see progress within the imaging field should common protocols or valid methods to integrate data be developed. To a degree, this has been done for the SPRING study (for which I have only analysed Cardiff data, outlined in Chapter 5) but to make stronger conclusions, this would need to be carried out on a much larger scale.

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Appendix

Figure 10.1 highlights the utility of using a GMM approach on a set of data comparing connectivity with placebo and connectivity with Ketamine. Panel A shows data without using the GMM procedure outlined in Chapter 2. The scatter plot of mean Z scores for correlation in placebo vs Ketamine (1) shows that most of the dots for Ketamine fall below the red line (y=x). This includes the weakest correlations which are likely to be noise rather than true network correlations. A paired t-test of correlation values between Ketamine and placebo is then plotted onto a connectivity matrix (2) and appears to show widespread reduced connectivity with Ketamine. This is unlikely and suggests that there is reduced SNR with Ketamine and therefore reduced correlations. Through plotting t-statistics of Ketamine versus placebo on a histogram (3), it appears that there is a normal null distribution but this is not centred on zero, suggesting that there is bias in the Ketamine-placebo comparison. The peak is also not symmetrical, but is wider on the right. Panel B shows the same data following the GMM procedure to remove "noise". The scatter plot (1) now shows that the weakest connections ($Z<2$) are clustered around the y=x line, suggesting the two sessions (active drug and placebo) have similar corrected null distributions. Following correction, those node-node connections that are known to be strongest from previous work (occipital and posterior parietal) show increased connectivity with active drug (2) and the t-statistic histogram now shows a reduced bias, with the peak closer to zero (3). GMM is therefore a useful procedure that allows us to model the null distribution for placebo and Ketamine sessions (or case and control groups) separately and correct correlation values to account for differences in nulls. This will then correct for any systematic errors, including SNR differences.

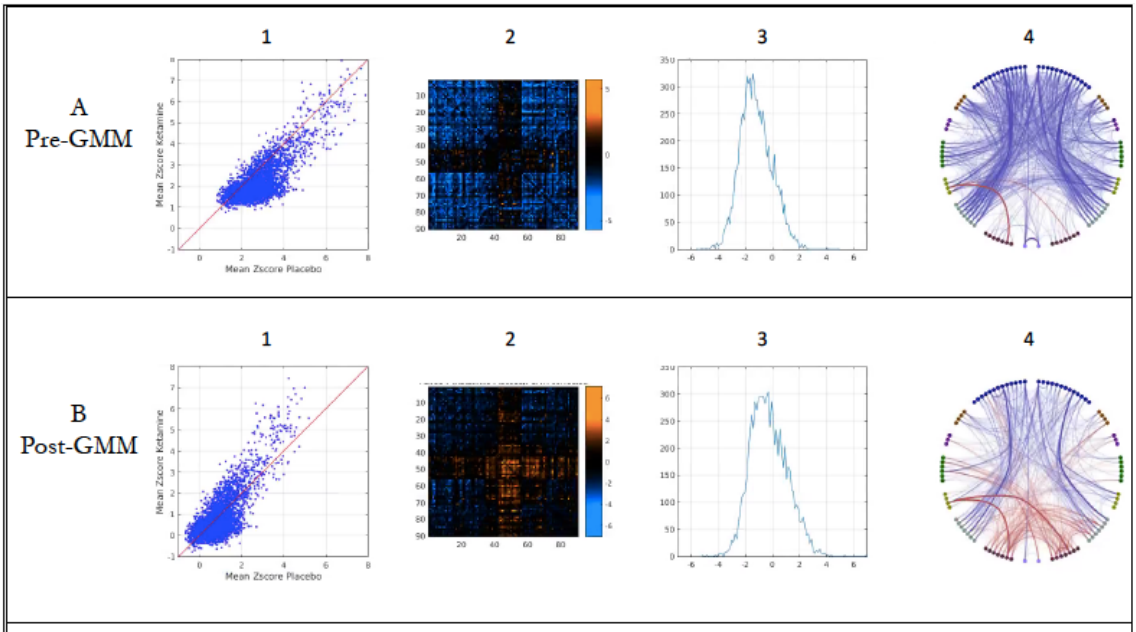

Figure 10.1 Comparison of data pre and post-GMM procedure

Panel A=Pre-GMM procedure
Panel B= Post-GMM procedure

1. Scatter plot showing uncorrected mean z-scores. X=control, y=case. Each blue dot represents one connection between AAL nodes.
2. Connectivity matrix showing t-test of connectivity between cases and controls. Blue=lower connectivity in cases. Orange = higher connectivity in cases.
3. Histogram of t-statistic values.
4. t-statistics plotted onto circular connectivity map to show spatial distribution of case-control effects.