Multimodal Imaging of the Salience Network in Schizophrenia

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Abstract

Growing evidence suggests that the Salience Network, anchored on the bilateral anterior insula and dorsal anterior cingulate cortex plays a key role in the pathophysiology of schizophrenia. However, several questions regarding the exact nature of these abnormalities in schizophrenia remain unanswered. This thesis examines the neurobiological basis of Salience Network dysfunction in patients with schizophrenia. Specifically, in this work, we use multi-modal neuroimaging techniques to investigate abnormalities in the functioning, connectivity and neurochemistry of the Salience Network in schizophrenia.

Chapter 1 introduces the concept of schizophrenia as a disorder of salience and the role of the Salience Network in this context. Chapter 2 describes participant recruitment, data collection and the neuroimaging techniques used in this work. Chapters 3 to 5 present the results from three research studies. In the first study, using functional Magnetic Resonance Imaging (fMRI) and Magnetoencephalography (MEG), we investigate regional brain activity in response to a task designed to engage the Salience Network. We report evidence demonstrating inefficient cerebral recruitment in patients with schizophrenia i.e. aberrant activity in task positive and task negative brain regions; along with reduced beta response to relevant stimuli in the Salience Network. These findings are reported in chapter 3. In chapter 4, we use high resolution 7T Magnetic Resonance Spectroscopy (MRS) and demonstrate that neurochemical abnormalities in schizophrenia vary between patients

depending on their clinical status. Specifically, we show that glutathione and glutamate concentrations in the Anterior Cingulate Cortex are prominently reduced in patients with residual schizophrenia. We also provide evidence linking two popular neurochemical theories of schizophrenia – the NMDA receptor hypofunction hypothesis and the theory of oxidative stress. Chapter 5 explores the question of disrupted effective connectivity within the Salience Network in schizophrenia using resting-state fMRI. In this chapter, we report abnormalities in effective connectivity within the Salience Network in patients with schizophrenia. We also demonstrate that these aberrant causal interactions are related to the neurochemical abnormalities reported in chapter 4. Finally, in Chapter 6, we discuss the significance and translational potential of these findings and offer recommendations for future work. Overall, using a multimodal imaging study design and a wide range of measures, this thesis reports novel findings which further our understanding of Salience Network dysfunction in schizophrenia and provides a good foundation for future research studies in this area.

Statement of Contributions

All data presented in this thesis were acquired as part of the Multi-modal Imaging Study in Psychosis (MISP) study led by Dr. Lena Palaniyappan, Professor Peter Liddle and Professor Peter Morris, jointly funded by the Medical Research Council (MRC), Dr. Hadwen Trust and the Wellcome Trust. Data collection was undertaken by a team comprising of PhD students, post-doctoral researchers and clinicians. I was involved in all aspects of data collection including recruitment and screening of healthy volunteers, liaising with clinicians to recruit patients, diagnosis consensus meetings, MRI and MEG data acquisition, cognitive testing and clinical interviews.

MEG data presented in chapter 3 were cleaned and pre-processed by other members of the team. However, subsequent analyses including all statistical analyses were undertaken by me. The fMRI analyses presented in chapter 3 and all analyses presented in chapter 4 and 5 were undertaken by me including data cleaning, processing and statistical analyses.

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Acronyms

ACC – Anterior Cingulate Cortex

AI – Anterior Insula

BOLD – Blood Oxygen Level Dependent

CEN – Central Executive Network

dACC – Dorsal Anterior Cingulate Cortex

DDD – Defined Daily Dose

dlPFC – Dorsolateral Prefrontal Cortex

DMN – Default Mode Network

DSM – Diagnostic and Statistical Manual of Mental Disorders

fMRI – Functional Magnetic Resonance Imaging

GCA – Granger Causality Analysis

Gln – Glutamine

Glu – Glutamate

GSH – Glutathione

MEG – Magnetoencephalography

mPFC – Medial Prefrontal Cortex

MRI – Magnetic Resonance Imaging

MRS – Magnetic Resonance Spectroscopy

NMDAR – N-methyl-D-aspartate Receptor

PCA – Principal Components Analysis

PCC – Posterior Cingulate Cortex

RM – Relevance Modulation

ROI – Region of Interest

SN – Salience Network

SOFAS – Social and Occupational Functioning Assessment Scale

SSPI – Signs and Symptoms of Psychotic Illness

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Chapter 1

Background and Aims

Our understanding of schizophrenia as a disorder and the neurobiological mechanisms involved has advanced considerably over the past few decades. For many years, schizophrenia was characterized as a neurochemical disorder with the primary abnormality being dopaminergic in nature. However, it is now recognized that aberrant dopaminergic neurotransmission does not alone account for the wide range of symptoms and dysfunction seen in patients with schizophrenia and that other neurochemical pathways are involved e.g. abnormalities in glutamatergic neurotransmission or impaired antioxidant defences. We also know now that schizophrenia is a disorder of cortical 'dysconnectivity' i.e. abnormal patterns of connectivity between various brain regions or networks. One of the most important findings in recent years has been the discovery of the Salience Network (SN) and the idea that schizophrenia is a disorder of aberrant salience detection. This introductory chapter will address the important neurochemical theories of schizophrenia and discuss how they might relate to salience abnormalities and the SN. Additionally, the role of the SN in salience processing and the evidence relating to connectivity abnormalities of the SN in schizophrenia will be addressed. Finally, this chapter will deliberate on the knowledge gaps in the literature and in this context, the research questions addressed in this thesis will be laid out.

1.1. Dopamine, psychosis and aberrant salience

The earliest evidence toward recognizing the nature of abnormalities in schizophrenia came from the use of chlorpromazine and subsequently other first generation antipsychotic agents to treat acute psychosis. The fact that all these drugs shared the common ability to block dopaminergic D2 receptors in the brain and reduce the synthesis of dopamine led to one the earliest theories of neurochemical imbalance in schizophrenia (Seeman & Lee, 1975). This concept was further supported by the fact that recreational drugs such as amphetamine, which increase dopaminergic activity in the brain, caused schizophrenia-like psychotic symptoms in healthy people and worsened psychotic symptoms in patients (Snyder, 1973; Angrist *et al*, 1980). This led to the development of the first dopamine hypothesis of schizophrenia, i.e. the theory of hyper-dopaminergia. This model stated that schizophrenia is an illness in which there is excessive dopamine release in the brain, leading to acute psychosis and a reduction in this disproportionate release of dopamine by the use of antipsychotics would lead to relief from these symptoms.

Although this idea of hyper-dopaminergia was generally accepted at the time, the primary issue with this hypothesis was the lack of relief from the negative symptoms and cognitive deficits after treatment with antipsychotics. This, coupled with an increasing interest in the syndrome-based model of schizophrenia, led to an inquiry into the idea that there might be more than one neurobiological process leading to the development of the illness (Crow, 1980). In 1991, Davis and colleagues published an important review paper in which they revised the dopamine hypothesis of schizophrenia. They used a

wide body of evidence, including evidence from Positron Emission Tomography (PET) neuroimaging studies to state that the imbalances of dopamine transmission could be different depending on the brain region and identified two areas, frontal and sub-cortical, with contrasting differences in dopaminergic function (Davis *et al*, 1991). The authors used the evidence that dopamine receptors display different brain distributions with D1 receptors found mainly in frontal areas and D2 receptors predominant in sub-cortical areas to hypothesize that schizophrenia is characterized by hypo-dopaminergia in the frontal D1 receptor regions, causing negative symptoms leading to a hyper-dopaminergic state in the sub-cortical D2 receptor areas, thereby causing positive symptoms (Davis *et al*, 1991). If a hypo-dopaminergia was the cause of negative symptoms, it was clear why antipsychotics which are dopamine antagonists had no or very little effect on these symptoms or in some cases even made them worse.

A considerable body of work was then undertaken using PET and Single Photon Emission Computed Tomography (SPECT) imaging in order to study various aspects of dopaminergic abnormalities in schizophrenia e.g. presynaptic dopamine levels, dopamine receptor binding, effects of treatment on dopamine receptors etc. These studies provided important evidence, some of which was of translational significance (Kapur *et al*, 2000; Pani *et al*, 2007). However, it was still unclear as to how dopaminergic abnormalities lead to the clinical expression of the disease. In an attempt toward delineating the relationship between dopamine and psychosis, in 2003, Kapur proposed the motivational or aberrant salience hypothesis of schizophrenia (Kapur, 2003). A few years prior to this, Laruelle and Abi-Dargham had published a review

article of neuroimaging studies in schizophrenia titled ‘Dopamine is the wind of the psychotic fire’ (Laruelle *&* Abi-Dargham, 1999). Kapur referred to this article in his paper and proposed a mechanism by which this could occur i.e. how dopamine could stoke the experience of psychosis (Kapur, 2003).

Before we discuss Kapur’s aberrant salience theory of schizophrenia, it is important to understand what we mean by salience. Salience can be defined as the quality of being particularly noticeable or important (Oxford English Living Dictionary, 2018). Essentially, if an event, object or even a thought or idea is prominent and attracts your attention, or is meaningful to you in some way, you would call it salient. Assigning appropriate salience to external and internal stimuli is extremely important, as in our everyday lives, we are often presented with multiple pieces of information from a variety of sources at the same time and deciding which of these to focus on makes a significant impact on our mental state. According to the motivational salience model, in the normal, healthy brain, dopamine mediates the process of assigning salience to external events and stimuli (Berridge *&* Robinson, 1998; Kapur, 2003). However in psychosis, there is abnormal stimulus-independent release of dopamine which leads to the aberrant assignment of salience to external and internal stimuli and events, which would not normally be regarded as salient, leading to psychosis. Kapur proposed that this framework was unique because under this model, aberrant saliences could persist and lead to psychosis even in the absence of sustaining stimuli (Kapur, 2003).

This aberrant salience hypothesis of schizophrenia proved to be influential because it provided an explanation for how the same illness (schizophrenia)

and the same set of symptoms (hallucinations and delusions) could manifest differently in different patients. According to this hypothesis, hallucinations arose from abnormal salience assigned to internal thoughts and memories while delusions were the result of a top-down cognitive effort made by a person in order to provide an explanation for these aberrant saliences. Thus, due to individual differences in each person's socio-cultural environment and experiences, various unique presentations of psychosis were observed in each patient. Additionally, the aberrant salience model also accounted for the divergent effects of antipsychotic medication in patients. According to Kapur, the psychological effect of antipsychotics was salience attenuation i.e. providing a platform for the dampening of these aberrant saliences in patients. However, relief from hallucinations and delusions would require further mental effort from the individual, which varies from patient to patient depending on their psychological make-up and other external influencing factors. Thus, essentially, psychosis could be regarded as a "dynamic interaction between a bottom-up neurochemical drive and a top-down psychological process" (Kapur, 2003).

This model was an important step forward in terms of understanding how dopaminergic abnormalities could lead to psychosis, however, it did not provide a full picture. Firstly, the aberrant salience model was a model of psychosis and not of schizophrenia. The model did not explain how dopaminergic abnormalities could result in negative symptoms and cognitive deficits. This was still a very important concern, since antipsychotic medication did not provide relief from negative symptoms or cognitive deficits for most patients. Kapur acknowledged this limitation in his paper and added

that several other factors, both genetic and environmental play an important role in the development of schizophrenia. Moreover, he also recognized the possibility that dysregulation of dopamine could be a secondary consequence of other neurochemical disturbances in schizophrenia. Building on this theory and on the basis of a wide body of evidence including studies investigating the prodromal phase and high risk subjects, Howes *&* Kapur published a paper on the third version of the dopaminergic hypothesis in schizophrenia. They hypothesized that the interaction of multiple factors or "hits" resulted in dopaminergic dysfunction, which they suggested was the "final common pathway to psychosis in schizophrenia" (Howes *&* Kapur, 2009). Furthermore, in this paper, Howes *&* Kapur provided an explanation for how dopamine dysregulation could lead to negative symptoms via the motivational salience model. They proposed that abnormalities in dopaminergic neurotransmission could lead to signals relating to rewarding stimuli being missed or "drowned out", and this lack of incentive salience could result in anhedonia and social withdrawal. Nevertheless, the authors also acknowledged that the evidence at present indicates that dopaminergic abnormalities seem to be primarily linked to psychosis and that if non-psychotic forms of schizophrenia were examined; it was plausible that they would not be related to dopaminergic dysregulation and that multiple other systems/pathways could be involved in the development of negative symptoms and cognitive deficits in schizophrenia (Howes *&* Kapur, 2009).

1.2. Beyond dopamine: NMDAR hypofunction and oxidative stress

Overall, it was clear that dopaminergic abnormalities played an important role in schizophrenia, and it was plausible that dopamine was the final common pathway that leads to schizophrenia. The aberrant salience hypothesis provided a good mechanistic framework which explained how neurochemical abnormalities lead to psychosis, and potentially also to negative symptoms. However, the lack of effective medication to provide relief from negative symptoms and cognitive deficits led researchers to explore other models of neurochemical dysfunction in schizophrenia. If other primary pathways were discovered, new interventions could be developed in order to target the primary abnormality as opposed to secondary dopaminergic deficits, and these could potentially provide relief from these treatment-resistant symptoms.

One of the neurochemicals that particularly attracted the interest of the scientific community was glutamate, the major excitatory neurotransmitter in the brain which plays an important role in controlling long-range communication. Glutamate mainly binds to three types of receptors, including the N-methyl-D-aspartate (NMDA) receptor (NMDAR). Initial interest in glutamate and the NMDAR was generated much in the same way as interest in dopamine. Psychotropic drugs such as ketamine and phencyclidine (PCP) which are NMDAR antagonists were seen to produce schizophrenia-like positive and negative symptoms in healthy individuals as well as stable patients (Javitt *&* Zukin, 1991; Malhotra *et al*, 1997; Lahti *et al*, 2001). This led to the theory that the blocking or hypofunction of NMDA receptors could lead

to aberrant glutamatergic neurotransmission and consequently schizophrenia. This hypothesis of glutamatergic dysfunction is known as the NMDA receptor hypofunction hypothesis of schizophrenia and was first proposed by Olney and Farber (Olney *&* Farber, 1995). According to their theory, a series of faulty NMDA receptors located on gamma-aminobutyric acid (GABA) inter-neurons leads to increase in glutamatergic neurotransmission which then causes excessive stimulation of post-synaptic neurons and ultimately leads to schizophrenia (Olney *&* Farber, 1995, Olney *et al*, 1999). Over recent years, the NMDA hypofunction model of schizophrenia has gained a lot of support and many researchers have focused on investigating how a primary NMDAR abnormality could lead to dopaminergic dysfunction and cause schizophrenia (Moghaddam *&* Javitt, 2012). One such theory focuses on two glutamatergic pathways, a mesolimbic pathway and a mesocortical pathway, with the same dysfunction in glutamatergic transmission affecting these two pathways differently, leading to different dopaminergic abnormalities i.e. excessive dopamine in the mesolimbic pathway causing positive symptoms and reduced dopamine in the mesocortical pathway contributing to negative symptoms and cognitive dysfunction (Stahl, 2007; Schwartz *et al*, 2012). It has also been suggested that NMDAR hypofunction may make the dopamine system more sensitive to the effects of psychological stress (Howes *et al*, 2015). However, according to Olney, Farber and colleagues, the interaction between the glutamatergic and dopaminergic system is the opposite i.e. with the dopaminergic system regulating the functioning of the NMDA receptors (Olney *et al*, 1999; Farber, 2003). Given the evidence demonstrating that traditional antipsychotics do not reverse ketamine induced psychosis or effectively treat

negative symptoms, it is apparent that the relationship between dopamine and NMDAR hypofunction is not straightforward (Lahti *et al*, 1995; Krystal *et al*, 1999). Therefore, it is most likely that the abnormalities in the dopaminergic and glutamatergic systems in schizophrenia are cyclical and inter-connected via complex mechanisms (Laruelle *et al*, 2003).

Another important neurochemical theory of schizophrenia which is very relevant in this context is the theory of oxidative stress (Wood *et al*, 2009a). Oxidative stress can result either from the excessive production of reactive oxygen species, e.g. due to inflammation, or from a reduction in oxidative defences, and can result in macromolecular damage of cell structures. Several research studies indicate that oxidative stress plays a role in the development of schizophrenia, although it is unclear whether or not it is a primary cause. Recent animal studies have suggested that there is a link between oxidative stress and NMDAR hypofunction (Steullet *et al*, 2006; Persson *et al*, 2006; Baxter *et al*, 2015). In their review article on this topic, Hardingham & Do make a convincing case for how these two processes might be reciprocally linked in the development of schizophrenia (Hardingham *&* Do, 2016).

Although NMDAR hypofunction is difficult to measure directly in humans, due to the development and continued use of in vivo neuroimaging techniques such as Magnetic Resonance Spectroscopy (MRS), we have been able to measure concentrations of glutamate and glutamine (a precursor to glutamate) in the brain and there is now a substantial body of evidence from MRS studies relating to glutamatergic abnormalities in patients with

schizophrenia (for a recent review see Poels *et al*, 2014). However, the exact nature of these abnormalities is still not fully understood due to inconsistencies in results between studies. Using MRS, it is also possible to measure oxidative damage in schizophrenia by examining the levels of glutathione (GSH), which is the brain's major antioxidant. Reduced levels of glutathione in schizophrenia have been reported (Do *et al*, 2000; Matsuzawa *et al*, 2008) and can indicate increased damage due to oxidative stress and neuroinflammation; however this is not always straightforward and some studies have also reported increased GSH levels in patients. At this point, several questions remain unanswered. What are the reasons for these inconsistencies? It is plausible that heterogeneity of the patient groups examined could have led to some of these divergent findings. Hence, do these abnormalities vary between patients depending on their clinical presentation? Are these abnormalities specific to particular brain regions or networks? Many studies have reported abnormalities in both glutamate/glutamine and glutathione in the anterior cingulate cortex (ACC), which is one of the key regions in the SN. This is discussed further in the following section 1.3 of this chapter. Additionally, given the hypothesis of a reciprocal link between oxidative stress and NMDAR hypofunction, is there a relationship between glutathione and glutamate concentrations in the brain? In order to effectively develop new treatments targeting NMDAR hypofunction or the antioxidant system, it is essential to answer these questions and we attempt to do this in chapter 4.

Overall, it is clear that dysfunction of a single neurochemical pathway cannot deterministically explain the diagnosis of schizophrenia, as it stands today.

Whether NMDAR hypofunction and oxidative stress are directly related to dopaminergic abnormalities is unclear. It is plausible that a complex interaction between these processes leads to multiple but separate aspects of dysfunction. Nevertheless, there is an agreement that neurochemical dysfunction in schizophrenia, particularly in the NMDAR system leads to cortical excitation-inhibition imbalance, which could affect the connectivity between various brain regions and networks. Dysconnectivity in schizophrenia, particularly in the SN and how it might relate to neurochemical imbalance is discussed in the following sections.

1.3. Dysconnectivity and the Salience Network

In 1911, Eugen Bleuler coined the term schizophrenia, roughly translated as “splitting of the mind” and noted that the primary and essential feature of the illness was a splitting of the psyche (Bleuler, 1911). Thus, the notion that a failure or loss of coherent integration between various mental functions is a key feature of schizophrenia is as old as the concept of schizophrenia itself. Schizophrenia is now widely recognized as a neural dysconnection syndrome, with the loss of connectivity between various brain regions or networks leading to the wide range of symptoms and dysfunction observed in patients (Friston, 2002; Stephan *et al*, 2009; Schmitt *et al*, 2011; Friston *et al*, 2016). The idea of schizophrenia as a disconnection syndrome was first identified by Friston *&* Frith in 1995. In their pioneering article, the authors suggested that while localized brain abnormalities can account for some of the symptoms observed in schizophrenia, these regional abnormalities alone do not sufficiently explain the varied clinical presentation of the illness. They put forth the view that this complex symptomatology observed in schizophrenia can be understood in terms of aberrant connections between various brain regions and systems. This dysfunctional integration between spatially distinct brain areas would physiologically manifest as abnormalities in ‘functional connectivity’ which could be measured using neuroimaging (Friston *&* Frith, 1995).

Functional connectivity, defined as a correlation between the time-series of two or more spatially distinct brain regions (Friston, 1994), or more simply put a correlation between spatially distinct neurophysiological events, can be

measured using various brain mapping techniques such as PET, Electroencephalography (EEG), Magnetoencephalography (MEG), task and resting-state functional MRI (fMRI). In recent years, an important development in the understanding of functional connectivity and integration in the brain in both health and disease has been the discovery of functional brain networks i.e. groups of brain regions which co-activate together during the performance of cognitive tasks and also during the resting-state (Biswal *et al*, 1995; Raichle *et al*, 2001; Greicius *et al*, 2003; Fox *et al*, 2005). Two important networks were identified by these studies. One was a primarily task-positive network known to play an important role in goal directed behaviour, decision making and top-down cognitive processing, now known as the Central Executive Network (CEN). And the other network was the default mode network (DMN), which was initially identified as a task-negative network important for introspective and self-referential processing, although recent reports suggest that the DMN also participates in certain types of tasks e.g. social cognition (Spreng *et al*, 2010; Mars *et al*, 2012). This finding allowed schizophrenia researchers to focus on abnormalities in connectivity within and between these key brain networks in patients (Garrity *et al*, 2007; Whitfield-Gabrieli *et al*, 2009; Tu *et al*, 2013; Littow *et al*, 2015).

However, in the context of schizophrenia research, the brain network which has garnered the most attention in recent years is the Salience Network, a system which plays an important role in detecting salient stimuli and events. Seeley *et al*. first used the term Salience Network, to describe a network anchored on the orbitofrontal insular cortices and the dorsal anterior cingulate cortex (dACC) involved in integrating sensory information with information

from other top-down sources in order to first detect and then make decisions relating to further processing of internal and external salient stimuli (Seeley *et al*, 2007). Although the use of the term Salience Network is recent, the idea that the insula and the dACC are involved in the processing of salient information has been around for much longer with studies demonstrating activation in these brain areas during the process of selectively attending to stimuli (Heinze *et al*, 1994; Hopfinger *et al*, 2000; Corbetta *&* Shulman, 2002; Krolak-Salmon *et al*, 2003; Booth *et al*, 2003). Studies have also shown abnormal activation patterns in these brain regions during selective attention and at rest in schizophrenia patients (Liddle *et al*, 1992; Carter *et al*, 1997; Weiss *et al*, 2003). Nevertheless, since the discovery of the SN, researchers have actively investigated abnormalities in its structure and function in schizophrenia, in order to explore the relationship between aberrant salience and SN dysfunction. Structural imaging studies using Voxel Based Morphometry Analysis (VBM) and meta-analyses of these studies have consistently reported grey matter abnormalities in the insula and ACC in patients with schizophrenia (Glahn *et al*, 2008; Fornito *et al*, 2009). Similarly, functional neuroimaging studies have also reported abnormalities in both SN functioning and connectivity in schizophrenia and their relationship with clinical symptoms (Pu *et al*, 2012; Walter *et al*, 2016; Pang *et al*, 2017). Interestingly, similar patterns of structural and functional abnormalities have also been observed in subjects at ultra-high risk for psychosis, indicating that the SN may be dysfunctional even before the onset of schizophrenia (Chan *et al*, 2011; Pelletier-Baldell *et al*, 2015; Wang *et al*, 2016a).

Another reason why the SN is such an important brain network is that it is known to enable the switch between different dynamic brain states, including the switch between the default mode and task-related brain states. In 2008, Sridharan and colleagues used Granger Causality Analysis (GCA), an effective connectivity technique, to demonstrate that the SN and the right insula in particular plays an important role in co-ordinating the functioning of and switching between the CEN and the DMN (Sridharan *et al*, 2008). While functional connectivity measures how correlated the activity is between two regions, effective or causal connectivity is a measure of the influence one neural system exerts over the other. Thus, by measuring effective connectivity, we can examine the causal relationships between brain regions and networks. The work done by Sridharan and colleagues was instrumental in our understanding how the SN controls the processing of salient information i.e. by modulating the functioning of the CEN which is involved in further processing of such stimuli and also by controlling activity in the DMN.

In 2012, based on a wide body of evidence from structural and functional neuroimaging studies of the SN, Palaniyappan *&* Liddle extended the aberrant salience model of schizophrenia and put forth the concept of proximal salience wherein an event or stimulus attains proximal salience when it produces a momentary state of neural activity within the SN which leads to the recruitment of other brain regions and modification or initiation of behavioural responses, if needed (Palaniyappan *&* Liddle, 2012). Thus, proximal salience differs from salience in so far as proximal salience additionally results in the SN-mediated engagement of other brain areas and prepares one for a behavioural response. Under normal circumstances, not all internal or

externally salient stimuli would achieve proximal salience. However, abnormalities in the SN could result in irrelevant stimuli being mistakenly regarded as proximally salient, leading to the recruitment of task positive brain areas, and consequently to hallucinations and delusions. On the other hand, SN dysfunction could also result in relevant stimuli not attaining proximal salience, and thus might not be processed further by higher-order cognitive control systems, leading to negative symptoms, cognitive deficits and consequently poor socio-occupational functioning. This model provided an excellent framework for understanding how a dysfunctional SN could lead to both the positive and negative symptoms of schizophrenia. The idea of proximal salience and the role of the SN in responding to such salient events tied in perfectly with the work done by Sridharan and colleagues, demonstrating the role played by the SN in switching between the CEN and DMN (Sridharan *et al*, 2008). Similar analyses using GCA were then undertaken to examine whether the effective connectivity between the SN and other networks is impaired in schizophrenia. In 2013, Palaniyappan and colleagues reported abnormalities in causal connectivity between the right anterior insula (AI) and the dorsolateral prefrontal cortex (dlPFC), a key node of the CEN in patients with schizophrenia (Palaniyappan *et al*, 2013). Two other studies using GCA have also found abnormalities in effective connectivity between the SN and the CEN and DMN in schizophrenia (Moran *et al*, 2013; Manoliu *et al*, 2014).

On the whole, a wide body of evidence indicates that the SN plays a critical role in the pathophysiology of schizophrenia. Nevertheless, some questions still remain unanswered. For instance, the effect of proximal salience on the

SN has yet to be investigated. The concept of proximal salience i.e. a momentary change in the activity of the SN which results in a change in the brain state has yet to be demonstrated using functional imaging methods. Before we attempt to fully understand the relationship between aberrant salience and SN dysfunction, it is important to recognize how salient and non-salient stimuli affect the brain. This is addressed in chapter 3 in the context of inefficient cerebral recruitment in schizophrenia. Neurochemical abnormalities clearly play an important role in schizophrenia, and at the moment, medication to target these abnormalities is the primary method of treatment for patients. Yet, mounting evidence indicates that schizophrenia is a dysconnection syndrome, with a crucial role played by the SN in modulating connectivity in the brain (Friston *et al*, 2016; Dong *et al*, 2018). Given that NMDAR hypofunction can cause abnormalities in connectivity due to aberrant glutamatergic neurotransmission, it is plausible that glutamatergic abnormalities are linked to connectivity disturbances in the SN. In fact, glutamatergic abnormalities have been consistently reported in the ACC (a key SN node) in MRS studies of schizophrenia, although the exact nature of these abnormalities is unknown due to inconsistencies in the literature. This issue is explored in chapter 4. Additionally, while abnormalities in causal connectivity between the SN and other brain networks in schizophrenia have been examined, there is some evidence which demonstrates that connectivity within the SN plays an important role in how the SN interacts with other brain regions (Jilka *et al*, 2014; Wang *et al*, 2016a). To our knowledge, no previous studies have investigated effective connectivity abnormalities within the SN in schizophrenia and hence this is one of the main aims of chapter 5. Relatedly,

another important research question which has yet to be answered is how SN connectivity abnormalities are related to neurochemical dysfunction in schizophrenia? If the SN nodes demonstrate abnormal glutamate concentrations (investigated in chapter 4), it is plausible that these abnormalities might be related to any effective connectivity abnormalities in the SN (investigated in chapter 5). Hence, correlations between neurochemical and effective connectivity abnormalities in the SN have also been examined in chapter 5.

1.4. Research questions and hypotheses

In summary, the research questions and hypotheses addressed in each chapter are as follows:

Chapter 3

What effect does proximal salience have on the SN and the brain? Do schizophrenia patients exhibit inefficient cerebral recruitment during the processing of salient and non-salient stimuli? These questions will be addressed using the Relevance Modulation (RM) task in fMRI and MEG.

Hypotheses:

- *Patients with schizophrenia demonstrate increased BOLD signal in the SN, CEN and other task-positive areas compared to healthy controls during the processing of relevant stimuli*
- *Patients with schizophrenia show a reduced beta response to task in the SN compared to healthy controls*

Chapter 4

Are there abnormalities in glutamate, glutamine and glutathione concentrations within the SN in patients with schizophrenia? Are there any differences in these neurochemical concentrations between patients depending on their clinical features? These questions have been investigated using single voxel MRS.

Hypotheses:

- *Glutathione, glutamate and/or glutamine concentrations in the ACC and/or left insula are reduced in all patients with schizophrenia compared to healthy controls, with a more prominent reduction in patients with residual schizophrenia*
- *There is a significant correlation between glutathione and glutamate concentrations in all three brain regions (ACC, left insula and visual cortex) across all subjects.*

Chapter 5

Are there effective connectivity abnormalities within the SN in schizophrenia? Additionally, are effective connectivity abnormalities related to neurochemical abnormalities within the SN? These questions are addressed using MRS and resting-state fMRI.

Hypotheses:

- *There are differences in effective connectivity patterns within the SN between patients with schizophrenia and healthy controls.*
- *There is a correlation between within-SN effective connectivity measures (mean Granger Causal influence) and neurochemical concentrations (ACC glutathione-glutamate component scores) in patients with schizophrenia.*

Chapter 2

Methodology

As mentioned previously, all data presented in this thesis were acquired as part of the Multi-modal Imaging Study in Psychosis (MISP) study led by Dr. Lena Palaniyappan, Professor Peter Liddle and Professor Peter Morris, jointly funded by the Medical Research Council (MRC), Dr. Hadwen Trust and the Wellcome Trust. This chapter includes the description of the patient and healthy volunteer cohorts recruited to this study, the clinical and cognitive testing on the day and the neuroimaging data acquired.

2.1. Participants

As the name suggests, the MISP study was designed in order to use complimentary neuroimaging modalities together, in order to develop a better understanding of the neurobiology of psychotic disorders. The broad aims of the study were: 1) to investigate neurochemical abnormalities in psychosis, 2) to examine the relationship between neurochemical abnormalities and co-ordinated brain activity, 3) to investigate the clinical continuum of psychosis i.e. similarities and differences between patients with schizophrenia and bipolar disorder in relation to these hypotheses. To this effect, and in line with a previous study conducted by our research group, the patient cohort recruited for this study included patients with schizophrenia, schizoaffective disorder and bipolar disorder with psychotic features. However, note that only

patients with schizophrenia and schizoaffective disorder were included in the analyses presented in this thesis.

Patients aged 18 to 55 years with a diagnosis, schizophrenia, schizoaffective disorder or bipolar disorder with psychosis were recruited to the study from inpatient and outpatient clinics and community services of NHS trusts in Nottinghamshire, Derbyshire and Lincolnshire, England. Patients were referred to the study by consultant psychiatrists and approached by a member of their mental health care team. Exclusion criteria were: 1) IQ below 70, 2) Lifetime history of substance dependence or harmful use in the past 6 months, 3) History of significant head trauma or medical conditions likely to have appreciable neurological or psychiatric effects, 4) Contraindications for MRI safety assessed by a standardized safety screening questionnaire, 5) Inability to speak English. Once a patient indicated an interest in participating in the study and eligibility criteria were satisfied, a consensus meeting with three trained study team members was undertaken in order to determine whether the patient satisfied the DSM-IV criteria for schizophrenia, schizoaffective disorder or bipolar disorder with psychosis. The consensus meetings were carried out in accordance with the best estimate procedure described by Leckman *et al* (Leckman *et al*, 1982) utilizing evidence regarding current clinical state and a retrospective review of case notes, and in consultation with the patient's psychiatrist and mental health care team, if required. All patients were scanned during a stable phase of illness, i.e. a change of no more than 10 points in their Social and Occupational Functioning Assessment Scale (SOFAS) score (defined in DSM-IV (American Psychiatric Association, 1994) between assessment 6 weeks prior to and

immediately prior to study participation. Most patients were receiving medication. The median Defined Daily Dose (DDD) (WHO-CCDSM, 2003) was calculated separately for antipsychotics, mood stabilizers including lithium and antidepressants and no patient had had a change in any of these medications six weeks prior to participating in this study.

Healthy participants with no personal or family history of psychotic disorders were recruited from the local community via posters. Other exclusion criteria were as for the patients. They were matched group-wise to the patient group for age (within 2 years), gender and parental socio-economic status (Rose *&* Pevelin, 2005).

2.2. Clinical and cognitive assessments

On the day of scanning, a number of assessments were undertaken in both the healthy volunteer and patient groups order to obtain information regarding current clinical features and cognitive functioning. Firstly, demographic data including age (and age at onset for patients) and gender were recorded using a demographics data sheet designed for this study. Additionally, information regarding nicotine, caffeine and recreational drug use was also recorded. Parental socio-economic status information was acquired and rated using the NS-SEC (National Statistic Socio-Economic Classification) scale for group matching purposes (Rose *&* Pevelin, 2005).

The question of matching groups appropriately has long been debated and has been discussed previously in a paper from our group (Kumar *et al*, 2015). In this study, we did not match groups using IQ since research studies suggest that lowering of IQ is related to the pathophysiology of schizophrenia, and also because variables such as age at onset of the disease, illness duration, and severity of symptoms are known to affect current IQ scores (Aylward *et al*, 1984; Woodberry *et al*, 2008) Instead, we used parental socio-economic status information in order to match the two groups. There is some evidence suggesting that parental socio economic status can impact brain development (Noble *et al*, 2005; Farah *et al*, 2006). Moreover, although it has been reported that parental occupational status is weakly associated with a risk of schizophrenia or severity of illness, the evidence is ambiguous (Parrott *&* Lewine, 2005). Therefore, we matched our groups for parental socio-economic status in order to minimize the risk of group differences in brain

development that are not directly related to the pathophysiology of schizophrenia. Nonetheless, to avoid potentially confounding effects of non-specific diffuse brain malfunction in cases with very low IQ, we excluded participants with a current IQ lower than 70, as measured by the Quick Test (Ammons & Ammons, 1962).

Handedness was assessed for both groups using the 12 items Annett scale (Annett, 1970). Social and occupational functioning was measured using the SOFAS – Social and Occupational Functioning Assessment Scale (SOFAS – DSM IV) (American Psychiatric Association, 1994). A clinical interview was also conducted by a trained team member using a standardized symptom assessment procedure (Signs and Symptoms in Psychotic Illness (SSPI) scale (Liddle *et al*, 2002a) in both groups. This interview was video recorded in order to ensure the consistency of symptom assessment and scoring, and in order to perform inter-rater reliability testing. IQ was assessed using the Quick Test (Ammons *&* Ammons, 1962). In order to measure cognitive functioning, two tasks – a modified version of the Digit Symbol Substitution Test (DSST) (Wechsler, 1981) which measures speed of processing and the Verbal Fluency test using two semantic fluency tasks (Kertesz, 1982; Mattis, 1988) were administered. In addition, the Thought and Language Index (TLI) was administered in order to measure formal thought disorder and disturbances in the fluency and form of thought (Liddle *et al*, 2002b). Moreover, healthy controls also completed the Cardiff Anomalous Perceptions Scale (CAPS), which is a measure of perceptual variances in non-clinical groups (Bell *et al*, 2006).

This study was approved by the West Midlands – Coventry & Warwickshire NHS National Research Ethics Service Committee (REC reference: 12/WM/0307). Informed consent was obtained from all participants and participants were made aware that they were free to withdraw from the study at any time. An inconvenience allowance was also provided. A total of 46 healthy controls, 41 patients with schizophrenia or schizoaffective disorder and 14 patients with bipolar disorder were recruited for this study.

2.3. Neuroimaging Techniques

The MISP study was conducted at the Sir Peter Mansfield Imaging Centre (SPMIC), University of Nottingham and all scans were acquired at the centre on the same day. The following sections describe the imaging modalities used in this study, the basic principles and theory behind these techniques and the neuroimaging data acquired as part of the study.

2.3.1. Structural Magnetic Resonance Imaging (sMRI)

Two structural MRI scans at ultra-high field (7T) were acquired as part of the MISP imaging protocol. Although this thesis does not report any detailed analyses of the sMRI data, the anatomical scans were used in order to accurately place the MR spectroscopy (MRS) voxels, and also for co-registration of the functional MRI (fMRI) and Magnetoencephalography (MEG) data.

The principles of Nuclear Magnetic Resonance (NMR) i.e. the phenomenon that when placed in a strong magnetic field, atomic nuclei absorb and re-emit radiofrequency energy provide the basis for the acquisition of MRI data (Rabi, 1938; Bloch *et al*, 1946; Purcell *et al*, 1946). Essentially, hydrogen protons in the water molecules in the human body possess a characteristic spin (called precession) and behave like small magnets. Hence, when placed inside an MRI scanner with a very strong magnetic field, these precessing protons align themselves in the direction of the magnetic field, B_0 and produce a net magnetization which is parallel to direction of the external magnetic field, called longitudinal magnetization. Since this longitudinal magnetization vector

is too small to be measured and detected by the MRI scanner, Radio Frequency (RF) pulses are then applied in order to flip the magnetized protons to the transverse plane and to bring them in to phase. This is known as transverse magnetization (Schild, 1990). When the RF pulse is switched off, the protons gradually lose phase coherence and return to their previous positions, parallel to B_0. During this process, the transverse magnetization decreases and the longitudinal magnetization increases in two independent processes (phenomena known as transverse (T2) and longitudinal (T1) relaxation respectively). As the T2 relaxation decreases, the protons emit a signal called the Free Induction Decay (FID). This signal can be measured by receiver coils in the MRI scanner. Phase and frequency encoding gradients are used in order to obtain spatial information and the FID signal is then reconstructed into an anatomical MRI image using Fourier transformation (Schild, 1990; Dale *et al*, 2015). Various pulse sequences are used in the acquisition of structural MRI data; however the most common sequences include spin echo pulse sequences (consisting of a 90 degree RF pulse, followed by one or more 180 degree refocussing pulses) and gradient echo sequences (in which a single RF pulse is used and magnetic field gradients are applied in opposing directions in order to refocus the spins) (Jackson *et al*, 1997; Dale *et al*, 2015). The time that it takes from delivering the first pulse to obtaining the echo or the signal is called time to echo or TE. The chosen pulse sequence is repeated a number of times in order to obtain the MRI image, and the time between two repetitions of the pulse sequence is known as the time to repeat or TR. These parameters are set at the time of planning the scan protocol and play an important role in determining the type of image

produced and the scan acquisition time (Dale *et al*, 2015). Although in principle the FID signal should be based on T2 relaxation times of the proton spins, in reality other factors such as magnetic field inhomogeneities affect the FID signal recorded by the scanner. This is generally not an issue if a spin echo pulse sequence is chosen, as the 180 degree refocussing pulses remove any effects caused by inhomogeneities in the magnetic field. However, in gradient echo pulse sequences, the observed rate at which T2 or transverse magnetization decays is affected by such inhomogeneities and hence is known as T2* (pronounced as T2 star) (Chavhan *et al*, 2009).

Structural MRI scans are very useful as they can provide detailed information allowing for the differentiation between tissue types i.e. grey and white matter. This distinction is achieved due to the differences in T1 and T2 relaxation times between different tissue types, which are reflected in the signal intensity differences in the MRI image. Thus, anatomical MRI images are usually either T1 or T2 (or T2*) weighted. Short TRs and short TEs are used in T1 weighted images, in order to maximize the differences in signal intensity between tissue types, due to differences in their T1 relaxation times and in order to ensure that differences in signal intensity due to differences in T2 relaxation times are not prominent. On the other hand, long TRs and long TEs are used to obtain T2 and T2* weighted images. Due to the long TR, longitudinal magnetization recovers fully and due to the long TE, differences in transverse relaxation between tissue types become more apparent, leading to T2 or T2* weighting (Schild, 1990).

Structural MRI has been widely used in neuroimaging studies of schizophrenia because of ease of data collection due to short acquisition times and the ability to acquire data in clinical settings. Grey matter loss in patients is one of the most widely reported findings in schizophrenia. Particularly of importance in relation to this thesis, meta-analyses of grey matter abnormalities in schizophrenia have consistently reported reductions in grey matter density in the insula and the anterior cingulate cortex (ACC), key nodes of the Salience Network (SN) in patients (Glahn *et al*, 2008; Fornito *et al*, 2009). Apart from measures of grey matter density and volume, sMRI scans have also been used to investigate patterns of cortical folding i.e. gyrification, cortical thickness and surface area in schizophrenia. Over the years, there has been a debate in terms of the difference between these measures and importance of selecting the appropriate phenotype in imaging studies (Hutton *et al*, 2009; Winkler *et al*, 2010). Other recent applications of sMRI include the use of graph theoretical measures to investigate grey matter network level abnormalities in schizophrenia and at-risk groups (Palaniyappan *et al*, 2015a; Tijms *et al*, 2015).

In the MISP study, a fast (3 minutes long) T1-weighted 3D MP-RAGE (rapid gradient echo) image was acquired at 7T in order to allow for the accurate placement of the MRS voxels and also for the co-registration of the MEG and fMRI data. Additionally, another longer (12 minutes long) T1-weighted scan called PSIR (Phase Sensitive Inversion Recovery) was also acquired. PSIR scans are high resolution images with improved tissue contrast. In clinical settings, PSIR can be used to detect cortical grey matter lesions and myocardial infarctions, which cannot be detected easily using standard T1-

weighted sequences (Huber *et al*, 2006; Sethi *et al*, 2012). These superior quality scans will be used for various structural data analyses such cortical gyrification, morphometry, network analyses etc.

2.3.2. Magnetoencephalography (MEG)

MEG is a neuroimaging technique that allows us to non-invasively study real-time cortical activity during various tasks and as well as at rest. MEG signals are representative of synchronous electrical currents generated in cortical circuits by the firing of pyramidal neurons. These electrical currents in turn produce orthogonally oriented low gradient magnetic fields around the head. Since the amplitude of these magnetic fields are very small tesla (10^{-15} T), MEG scanners use superconducting quantum interference devices (SQUIDs) which are capable of mapping this magnetic field (Cohen, 1972). These SQUIDs are cooled using liquid helium and connected to sensors – either magnetometers or gradiometers inside the MEG helmet which record the magnetic field information. Sensor data are then used to deduce the location of the electrical current source in the brain (Hari *&* Salmelin, 2012; Singh, 2014). This information is then used in conjunction with anatomical MRI images, in order to investigate hypotheses relating to the functioning of brain regions and networks. In the MISP study, we used a CTF MEG scanner (MEG International Services Ltd., Coquitlam, Canada) with a synthetic third order gradiometer configuration and 275 sensor channels. Although magnetometers are superior in terms of signal quality and the ability to map sources from deeper brain regions, synthetic gradiometers provide excellent noise cancellation and a better signal to noise ratio (Vrba *&* Robinson, 2001; Singh,

2014). This is extremely important because interference from outside magnetic sources of noise can highly affect the MEG signal, as the amplitude of the magnetic field produced by such sources is considerably larger than the magnetic field produced around the head as a result of neural activity. Due to this reason, the MEG system is placed inside a magnetically shielded room.

In this study, using MEG, we aimed to investigate the differences in evoked cortical activity between patients and healthy volunteers in response to two tasks and also at rest. The first task was a simple visuomotor task in which participants have to press a button multiple times when a particular stimulus appears on the screen. The results from this task have been published (Robson *et al*, 2016) and have not been reported in this thesis. The second task is called the Relevance Modulation (RM) task which was designed to study the activity of the SN. In this task, participants are shown two sets of alternating stimuli – butterflies and ladybirds and are asked to focus on one of these in each block, making one set of stimuli relevant and the other irrelevant for any given block. The task itself is a target detection task where participants have to detect relevant target stimuli in each trial and press a button when the target appears. The analysis of this task has been reported in chapter 3.

The main advantage of MEG is its excellent temporal resolution which enables us to study cortical activity at a millisecond timescale. Additionally, since the magnetic field can pass through the brain and skull without any interference, MEG offers a superior method of studying cortical activity compared to other electrophysiological techniques like Electroencephalography (EEG). MEG is also a direct measure of cortical

activity and thus, brain function, unlike measures such as fMRI which are only indirect and secondary measures. Nevertheless, certain limitations of MEG must also be taken into account. Firstly, MEG can only detect signals originating from the firing of pyramidal neurons which are tangential to the head surface, and cannot detect signals from radial dipoles, unlike EEG which is also sensitive to radial dipoles. EEG is also sensitive to deeper brain regions; possibly on account of volume conduction of the EEG signal (Hamalainen *et al*, 1993). Additionally, due to the costs associated with maintaining the SQUIDs, MEG scans are expensive and hence less likely to be used commonly in clinical practice. However, the most widely discussed issue is relating to the analysis of MEG data and the 'inverse problem' i.e. using the measured magnetic field information from outside the brain, how can we accurately determine the source or location of neural activity in the brain? The inverse problem is complex and ill-posed because it does not offer a single unique solution. Because there are multiple possible answers to the problem, several assumptions are made in order to attempt to find the best possible solution. Various approaches have been used to answer the inverse problem including localized methods like modelling using an equivalent current dipole (ECD) (Hari, 1991) and distributed methods such as minimum norm or minimum current estimates (MNE/MCE) (Hamalainen *&* Ilmoniemi, 1994; Uutela *et al*, 1999). However, in the analyses reported in this thesis, we use another commonly used source localization technique called beamforming, which uses a spatial filtering method and is based on the estimated weighted sum of all the sensors, 275 in our case. Essentially, the beamformer calculates and preserves the signals originating from

predetermined spatial locations in the brain and thus, this method can be sequentially applied to the whole brain to produce a map of cortical activity (Hillebrand *&* Barnes, 2005; Brookes *et al*, 2008). However, as with other methods that attempt to provide solutions to the inverse problem, beamforming makes certain assumptions. Since the spatial filtering is based on estimating signals from a specific location and attenuating signals from other areas, one main assumption it makes is that signals originating from spatially distinct areas are independent. This assumption is a significant limitation as the beamformer will recover very little power if it encounters persisting, correlated neuronal sources. However, recently, modifications to this model have been suggested in order to accurately map spatially distinct but temporally correlated sources (Brookes *et al*, 2007). Overall, beamforming has proven to be a useful technique in the analysis of MEG data, particularly in terms of detecting induced changes in oscillatory amplitude even in cases where a strong average signal is not produced (Hillebrand *&* Barnes, 2005).

As reported by Uhlhaas & Singer in their review paper on this topic, abnormalities in neural oscillations and synchrony play a crucial role in the pathophysiology of schizophrenia (Uhlhaas *&* Singer, 2010). Although EEG has been used to investigate abnormalities in neural oscillations in schizophrenia and other psychiatric disorders for years, the use of MEG in psychiatric research is now becoming increasingly popular. Using MEG, our understanding of gamma band abnormalities in schizophrenia have considerably improved (Tsuchimoto *et al*, 2011; Uhlhaas *et al*, 2011). Additionally, our research group has demonstrated abnormalities in the beta band within SN regions during the processing of task relevant stimuli in

schizophrenia patients (Liddle *et al*, 2016). The MEG analyses presented in chapter 3 have been based on this work. New approaches are also being developed to measure connectivity within and between networks using MEG (Kim *et al*, 2016; Brookes *et al*, 2016). Moreover, multi-modal imaging study designs such as the MISP study allow us to take advantage of the excellent temporal resolution of MEG and combine that with the excellent spatial resolution of fMRI. Given its advantages, MEG offers enormous potential in terms of discovering disease or symptom-specific phenotypes of oscillatory activity in networks such as the SN which play an important role in the pathophysiology of schizophrenia. Such work could aid our understanding of the mechanistic process that leads to illness onset and progression, and thus to the development of new interventions.

2.3.3. Magnetic Resonance Spectroscopy (MRS)

Magnetic Resonance Spectroscopy (MRS) is a powerful imaging technique that allows us to non-invasively measure the concentrations of various metabolites in the brain. As with MRI, MRS is also based on the principles of NMR. MRS can be used with different nuclei but the most commonly used form is ^{1}H MRS, also known as proton MRS. ^{1}H MRS works by examining the magnetic resonance properties of hydrogen nuclei attached to other molecules. Since differences in molecular structure and chemical environment can affect the surrounding magnetic field, the resonance frequencies for hydrogen protons attached to different molecules vary. This shift in the resonance frequency is called chemical shift, measured in ppm (parts per million). This chemical shift information is used to output a collection of peaks,

representing the differences in the hydrogen nuclei in different chemical environments, called the spectrum (Bluml, 2013). The spectrum typically consists of metabolite peaks such as N-acetyl aspartate (NAA), Creatine (Cr), Choline (Cho), Myoinositol, Glutamate (Glu), Glutamine (Gln) or both together (Glx) and Gamma-Aminobutyric Acid (GABA), which can be detected by MRS. However, metabolite quantification is not always straightforward. One important factor which determines the observable peaks in the NMR spectra is J-coupling or spin-spin coupling, which describes the magnetic interactions between neighbouring proton spins. A proton can thus experience two or more different magnetic environments, leading to signal splitting and multiple peaks e.g. doublets or triplets. This phenomenon and other factors such as low metabolite concentrations and overlapping peaks make it difficult to accurately quantify metabolite concentrations. For instance, in the context of schizophrenia research, Glu and Gln are important but it is often difficult to distinguish between the two at 3T or lower magnetic field strengths and hence many studies report Glx concentrations. Additionally, neurochemicals such as dopamine are virtually undetectable using MRS. One study used group averaged spectra to report reduced dopamine in the substantia nigra in Parkinson's disease (Groger *et al*, 2014); however this procedure is uncommon and requires empirical testing.

Similar to sMRI, the choice of TE and TR affects the signal and the number of peaks which are observable in the spectra. If a long TE is used, the signals from metabolites with shorter T2 relaxation times decay and only the peaks from metabolites with longer T2 relaxation times are visible on the spectra. One advantage of a long TE is that due to their small number, the peaks can

be easily separated. On the other hand, if shorter TEs are used, multiple peaks are observable on the spectra and hence we can quantify more metabolites, especially those with short T2 relaxation times like glutamate. However, the disadvantage is that some peaks may be overlapped by others. Similarly, when longer TRs are used, fully relaxed spectra can be obtained whereas shorter TRs can lead to saturation effects with shorter peaks. Nevertheless, longer TRs are often impractical to use due to problems with prolonged acquisition times, particularly in clinical settings. Hence, TRs between 1500-2500 ms are widely regarded as an accepted compromise.

^{1}H MRS data are acquired by first shimming the magnetic field around the region of interest in order to account for inhomogeneities. In larger regions of interest, it is more difficult to achieve a homogenous magnetic field, however this process is extremely important as accurate identification of metabolite peaks is impossible in a heterogeneous magnetic field due to dispersion of the resonance frequencies (Juchem *&* de Graaf, 2017). Another important step in the acquisition of spectra is water suppression. As the hydrogen signal from water is large, water suppression is essential in order to be able to discern the peaks of the neurometabolites. In this study, the main aim was to investigate neurochemical concentrations in specific parts of the brain which are implicated in the pathophysiology of schizophrenia. To this effect, we acquired ^{1}H MRS data from three voxels of interest – the left insula, the ACC and the visual cortex using Single Voxel Spectroscopy (SVS). SVS works by optimising parameters to measure metabolite concentrations only from pre-determined regions of interest. This approach is preferred if there are apriori hypotheses relating to specific brain areas, since acquisition times are shorter

for SVS as compared to Chemical Shift Imaging (CSI) which is a multi-slice/volume technique suitable for mapping large areas. As with sMRI, different pulse sequences can be used to acquire MR spectra. The most commonly used pulse sequences for ^{1}H MRS are STEAM (STimulated Echo Acquisition Mode) and PRESS (Point RESolved Spectroscopy). In this study, we used the STEAM sequence due to its advantages over PRESS such as the ability to examine metabolites with short T2 decays by using short TEs and reduced chemical shift artefacts due to improved RF bandwidth (Moonen *et al,* 1989; Bluml, 2013). Although PRESS offers increased signal-to-noise ratio (SNR), since the ^{1}H MRS data in this study were acquired using a high resolution 7T MR scanner (which offers a significantly better SNR and increased spectral resolution for localized MRS), STEAM was chosen over PRESS.

Pre-processing steps for ^{1}H MRS include reformatting, realignment and phase-correction followed by fitting the data to model spectra in software packages such as LCModel (Provencher, 1993) or jMRUI (Stefan *et al*, 2009). Metabolite concentrations are then computed and normalized to either another metabolite such as creatine or NAA, or to internal water. Internal metabolite referencing has been used widely; however, it is not always an appropriate choice in clinical studies where concentrations of these metabolites (e.g. creatine) may be affected in patient groups. In such situations, unsuppressed water or external references or phantoms are more suitable (Near, 2014). Nevertheless, absolute quantification of metabolite concentrations is complicated and several factors such as metabolite and

water T1 and T2 relaxation times, partial volume effects, etc. need to be taken into account (Jansen *et al*, 2006).

^{1}H MRS has been extensively used over the past couple of decades in schizophrenia research (for a recent review see Wijtenburg *et al*, 2015). Altered NAA, glutamate, glutamine and GABA concentrations have been widely reported in patients with schizophrenia and recently there has also been an increasing interest in glutathione (GSH) abnormalities in schizophrenia. Yet, there are inconsistencies between studies with some reporting an increase and others reporting a decrease or no changes in these neurochemicals in patients. This issue has been discussed in chapter 4. In summary, MRS is a promising technique and when used in combination with other imaging modalities such as fMRI (as reported in chapter 5), it can allow us to explore the relationship between neurochemical dysfunction and connectivity abnormalities in schizophrenia and other disorders. Moreover, technical advancements in MRS research such as improved pulse sequences with shorter acquisition times, new spectral editing and quantification techniques (Soher *et al*, 2014) and development of approaches without water suppression (Dong, 2015) will potentially allow for MRS to be used in drug development and clinical practice in the future.

2.3.4. functional Magnetic Resonance Imaging (fMRI)

fMRI is another non-invasive imaging technique that can be used to study brain activity. This technique is centred on the concept that when any given part of the brain is active or in use, its oxygen and glucose consumption

increase and in order to replenish these, there is an increase in oxygenated blood flow to that region due to neurovascular coupling. The degree of oxygenation in different parts of the brain would thus vary depending on the neural activity in various brain regions and thus by mapping the flow of oxygenated blood in the brain, it is possible to map neuronal activity. This process works due to the differences in the magnetic properties of oxygenated and deoxygenated haemoglobin. As described in the pioneering article by Ogawa and colleagues, oxygenated blood is diamagnetic and hence doesn't affect its surrounding magnetic field and there is no signal loss in areas with oxygenated haemoglobin. However, when diamagnetic oxygenated haemoglobin gives up its oxygen, the resulting deoxygenated haemoglobin is paramagnetic i.e. it is attracted by and hence distorts the surrounding magnetic field, resulting in a loss of signal. Therefore, differences in venous blood oxygenation affect the T2* decay, which takes into account inhomogeneities in the magnetic field. Thus, these differences in signal intensities as an effect of differences in blood oxygenation can be measured using T2* weighted MRI (Ogawa *et al*, 1990). This is known the Blood Oxygenation Level Dependent (BOLD) effect and is the basis of fMRI.

The response measured using fMRI is known as the haemodynamic response (HDR). Following neural activity in a given area, there is reduced blood oxygenation in that area due to the consumption of energy and this is results in an initial dip in the HDR. Immediately after that, blood oxygenation levels in that area increase due to incoming oxygenated blood in order to compensate and meet the energy demands. However, the incoming oxygenated blood exceeds the demand and due to this, the amount of oxygenated blood

increases and deoxygenated blood decreases after neural activation. After 4-6 seconds, peak blood oxygenation levels are attained and then if no further neural activity takes place, then the levels go back to baseline and typically undershoot before returning to baseline again. The estimated shape of the HDR is usually modelled using a canonical haemodynamic response function (HRF).

fMRI scans are typically acquired using Echo Planar Imaging (EPI) which allows for a fast acquisition as well as high SNR (Mansfield, 1977) and analysed using software such as SPM, FSL, AFNI etc. Standard fMRI data pre-processing steps include slice-timing correction, re-alignment, co-registration, movement correction and removal of nuisance covariates, normalization and smoothing. For resting-state data, additional steps such as scrubbing and filtering are also recommended. In the MISP study, we acquired two fMRI scans – one during the performance of the RM task (described in section 2.3.2. above) and one at rest using multi-slice gradient EPI sequences at 7T. Although fMRI has excellent spatial resolution, its temporal resolution is quite poor due to delay or lag between neural activation and measured signal. Hence, by acquiring both fMRI and MEG data using the same RM task, we can take advantage of both the excellent spatial of fMRI as well as the superior temporal resolution offered by MEG to investigate differences in SN activity during the processing of relevant and irrelevant stimuli between patients and controls. This analysis is reported in chapter 3. fMRI can not only be used to measure regional brain activity, but can also be used to investigate connectivity in the brain. A large body of evidence suggests that schizophrenia is a ‘dysconnection’ syndrome and several of

these research studies have used fMRI to investigate abnormalities in functional connectivity (instantaneous correlations in activity between spatially separate brain regions) as well as effective connectivity (causal interactions in which neural activity in a given region can predict neural activity in another region) in patients with schizophrenia (for reviews see Gur *&* Gur, 2010; Ruiz *et al*, 2013). Chapter 5 includes an analysis of abnormalities in effective connectivity within the SN in schizophrenia patients using Granger Causality Analysis (GCA). Other planned fMRI analyses with the MISP dataset include the use of Dynamic Causal Modelling (DCM), another effective connectivity technique to investigate causal interactions between SN and CEN nodes during the performance of the RM task in patients and controls.

Although fMRI is a popular technique, there have been considerable debates over the years with regards to the neural origins of the BOLD signal. This is a crucial question because understanding where the BOLD signal comes from is important in order to accurately interpret results from fMRI studies. Initially, the BOLD response was thought to be correlated to neuronal firing or action potentials. Subsequent research studies confirmed that BOLD responses were more closely related to local field potentials (LFPs) which reflect post-synaptic regional neural activity. Thus, BOLD was proposed to be an indicator of the input to a group of neurons and their intrinsic processing (Logothetis *et al*, 2001; Logothetis, 2002). However, there is also considerable evidence which suggests that the BOLD response does not just measure local neuronal activity, but that other top-down modulatory factors such as stimulus anticipation and response preparation also affect the measured fMRI signal (Kastner *et al*, 1999; Silver *et al*, 2007; Sirotin *&* Das, 2009). Nevertheless,

due to the indirect nature of the fMRI signal (the HDR is only a surrogate measure of the activity of neuronal populations), a complete understanding of the complex mechanisms that lead to the BOLD response under task or resting conditions is still elusive. Apart from being an indirect measure of neuronal activity, fMRI also suffers from other disadvantages such as poor temporal resolution, inability to distinguish between excitatory and inhibitory activity and susceptibility to movement and physiological artefacts. Despite these drawbacks, fMRI is a non-invasive method of studying brain activity, offers excellent spatial resolution and data acquisition is easy as fMRI scans be performed at the same time as a conventional anatomical MRI scan. Hence, fMRI is still widely used in research and is also being used in clinical practice e.g. pre-surgical neural activity and network mapping for epilepsy or tumour surgery patients (Bookheimer, 2007; Liu *et al*, 2009). New technological developments continue to emerge each year in terms of novel fMRI contrasts, faster acquisition sequences, improved data processing techniques etc. and despite other non-invasive imaging modalities like MEG gaining popularity, fMRI will likely continue to be used in psychiatric research in the foreseeable future.

Chapter 3

Inefficient recruitment in schizophrenia: the role of the Salience Network

3.1. Abstract

Recent research has suggested that the dysregulation of key brain networks such as the Central Executive Network (CEN), Default Mode Network (DMN) and the Salience Network (SN) play a cardinal role in the pathophysiology of schizophrenia. This work explores the function of the SN during the processing of relevant and irrelevant stimuli and its role in the efficient functioning of other networks and the brain as a whole. In this analysis, we aimed to test the hypothesis that inefficient cerebral recruitment during the processing of relevant stimuli in schizophrenia patients would manifest as increased BOLD signal in SN and CEN regions in fMRI; and reduced beta response to task in the SN in MEG. 38 healthy volunteers and 18 patients with schizophrenia were included in this analysis. 7T fMRI and MEG data were acquired during the performance of a target detection paradigm with relevant and irrelevant stimuli designed to test the effects of proximal salience on the brain. Analysis of fMRI data revealed that patients with schizophrenia demonstrated an increased BOLD response in CEN areas and decreased BOLD in DMN areas during the processing of relevant vs irrelevant stimuli compared to healthy controls ($p < 0.05$). MEG analysis demonstrated that

patients also showed a reduced beta response to relevant stimuli in the SN ($p < 0.05$). These findings suggest that patients with schizophrenia have to expend additional efforts to suppress attention to the internal world, resulting in inefficient recruitment of both task positive and negative brain areas during the processing of salient information.

3.2. Introduction

Over two decades ago, Friston and Frith put forth the view that the composite range of symptoms and dysfunction observed in schizophrenia can be understood in terms of aberrant connections between various important brain regions and systems (Friston *&* Frith, 1995). These ideas led to the generation of the 'dysconnection hypothesis' of schizophrenia. A growing body of evidence from neuroimaging studies in recent years has supported this hypothesis and proposed that the dysregulation of brain networks such as the Central Executive Network (CEN), Default Mode Network (DMN) and the Salience Network (SN) play a cardinal role in the pathophysiology of schizophrenia. Hence, there has been a renewed focus on investigating the precise nature of abnormalities in the functioning of these key networks in order to fully understand the neurobiological underpinnings of the complex nature of impairments observed in patients. Abnormalities in network function in schizophrenia have been reported by several studies and many of these relate to the CEN, which is known to be involved in top-down cognitive control and is active during tasks which require sustained attention, goal-directed decision making and the working memory (Corbetta *&* Shulman, 2002; Dosenbach *et al*, 2007; Niendam *et al*, 2012). Although studies using functional magnetic resonance imaging (fMRI) have shown both hypo-activation and hyper-activation of the nodes of the CEN during task performance in patients with schizophrenia, recent research has shown that when schizophrenia patients perform at the same level as healthy controls, they exhibit increased blood-oxygen-level dependent (BOLD) activity in CEN brain regions such as the dlPFC (Callicott *et al*, 2003; Potkin *et al*, 2009; Jiang

et al, 2015). This excessive recruitment has been thought to be a result of physiological inefficiency in schizophrenia due to which patients need to recruit additional resources in order to achieve the same level of performance as healthy controls (Potkin *et al*, 2009; Jiang *et al*, 2015). It has also been hypothesized that this hyperactivity reflects reduced efficiency with which the nodes in this network communicate with each other, and potentially with other brain networks (Ramsey *et al,* 2002). Similarly, several studies have also reported inefficient DMN functioning in schizophrenia (for a review see Whitfield-Gabrieli *&* Ford, 2012). The DMN - comprised of the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC) extending into the precuneus and the lateral parietal cortex is a network which is active during rest and involved in task-independent and self-referential processing (Biswal *et al*, 1995; Raichle *et al*, 2001). In healthy controls, BOLD activity in the DMN is typically reduced during task performance, an indicator of decreased internal processing and increased engagement with external stimuli (Daselaar *et al*, 2004; Shulman *et al*, 2007). Various studies have reported reduced DMN suppression during task performance in patients with schizophrenia (Pomarol-Clotet *et al*, 2008; Whitfield-Gabrieli *et al*, 2009; Anticevic *et al*, 2012; Fryer *et al*, 2013), however opposite effects have also been reported (Harrison *et al*, 2007; Mannell *et al*, 2010). fMRI data show that activity in the CEN and DMN is typically anti-correlated, with the CEN active during task performance and the DMN active at rest, and Liddle *et al*. have suggested that inefficient cerebral recruitment in schizophrenia could manifest as abnormal activation of task positive regions but also as inappropriate

activation in task negative regions which could interfere with task performance (Liddle *et al*, 2013).

A substantial body of work indicates that it is the SN which mediates the switching between these two key networks i.e. the CEN and the DMN (Sridharan *et al*, 2008; Deshpande *et al*, 2011; Goulden *et al*, 2014; Chand *&* Dhamala, 2016) and thus plays a key role in co-ordinating brain function. The primary role of the SN (comprised of the bilateral anterior insula (AI) and the dorsal anterior cingulate cortex (dACC)) is the bottom-up detection of novel salient stimuli. Menon *&* Uddin proposed that once a salient stimulus is detected, the SN then facilitates further processing of the stimulus by engaging areas of the brain involved in attention, working memory and cognitive control (i.e. CEN regions) and disengages brain areas that are not immediately task relevant (e.g. DMN regions) (Menon *&* Uddin, 2010). In 2012, Palaniyappan *&* Liddle proposed an alternate view, which is that the SN is involved in a more general 'switching' i.e. recruitment of brain regions required to process a current salient stimulus, while suspending the processing of a previously salient stimulus (Palaniyappan *&* Liddle, 2012). This takes into account the possibility that both the CEN and the DMN might be engaged in tasks which require integration of internal thoughts and external stimuli. Essentially, it is therefore plausible that inefficient recruitment of the CEN and DMN in schizophrenia is the result of a dysfunctional SN, and several studies have consistently reported both structural and functional abnormalities within the SN in schizophrenia (Glahn *et al*, 2008; Palaniyappan *et al*, 2012; Uddin, 2015). If the SN is inefficient, irrelevant stimuli could be mistakenly regarded as salient, and this aberrant salience could lead to

perceptual distortions and positive symptoms such as hallucinations and delusions (Kapur *et al*, 2003). Palaniyappan *&* Liddle extended this aberrant salience model and put forth the concept of proximal salience wherein an event or stimulus attains proximal salience when it produces a momentary state of neural activity within the SN which results in a change in the brain state and leads to initiation or modification of responses, if needed (Palaniyappan *&* Liddle, 2012). Thus, abnormalities in the SN could also result in relevant stimuli not attaining proximal salience, and thus might not be processed further by higher-order cognitive control systems (e.g. CEN areas), leading to negative symptoms, cognitive deficits and consequently poor socio-occupational functioning. In line with this, recent studies have demonstrated abnormal patterns of causal connectivity between the insula and CEN as well DMN nodes in patients with schizophrenia (Palaniyappan *et al*, 2013; Moran *et al*, 2013; Manoliu *et al*, 2014). Additionally, these aberrant patterns of causal connectivity were correlated with clinical features and/or cognitive deficits in all three studies.

Overall, although these results reinforce the aberrant salience model of schizophrenia and also suggest that there is a link between aberrant salience and inefficient cerebral recruitment, the question of inefficient recruitment in the context of the SN dysfunction in schizophrenia has not been directly investigated. Moreover, it is yet unclear as to how the SN and subsequently other brain areas process stimuli that are salient as opposed to those that are not. Most previous studies have used a range of cognitive tasks to explore the functioning of the SN and other brain networks. However, the nature of these tasks have not allowed for a study of the direct effect of proximal salience on

the brain, due to confounding effects of task related motor responses. In order to fully comprehend the nature of SN dysfunction in schizophrenia and its effect on both inefficient recruitment of and dysconnectivity between brain networks, it is crucial to investigate how proximal salience can modulate the functioning of the SN. Therefore, a target detection task was with minimal motor responses called the Relevance Modulation (RM) task was developed in which the behavioural salience of stimuli was influenced by alternating relevant and irrelevant images that were visually identical (Brookes *et al*, 2012; Brookes *et al*, 2015; Liddle *et al*, 2016). This task was specifically designed to differentiate patterns of brain activity produced by relevant and irrelevant stimuli, even when no motor response is required or executed, thus being able to directly investigate the effect of proximal salience on the brain. The RM task was first used in a Magnetoencephalography (MEG) study in healthy controls in order to investigate changes in beta amplitude in brain networks as a function of task relevance (Brookes *et al*, 2012). In a follow-up study, this task was used to compare differences in beta amplitude in the SN between schizophrenia patients and healthy controls (Liddle *et al*, 2016). A priori hypotheses were formulated for the beta band because multiple studies have reported beta band abnormalities in schizophrenia and it has been proposed that these are likely to reflect disrupted long-range connectivity and integration of information across networks (Schnitzler *&* Gross, 2005; Donner *&* Siegel, 2011).

The first study in healthy controls showed initial event-related desynchronization (ERD)) i.e. reduction in beta amplitude and subsequent event-related synchronization (ERS) i.e. increase above baseline during both

relevant and irrelevant trials in the salience network and the left motor network, although these changes in beta amplitude were more prominent during relevant trials (Brookes *et al*, 2012). The follow-up patient study demonstrated that while the ERS in healthy controls was significantly enhanced for relevant trials compared to irrelevant trials, the pattern of ERD-ERS was more exaggerated in the irrelevant trials in schizophrenia patients (Liddle *et al*, 2016). Since the beta band is involved in long-range integrative processes, these results were interpreted by the authors as a failure of the SN to detect and signal the salience of task-relevant stimuli to other networks, leading to aberrant processing of these relevant stimuli. A similar process could also account for the incorrect attribution of salience to irrelevant stimuli and aberrant processing of these stimuli, in line with the aberrant salience model (Kapur *et al*, 2003; Palaniyappan *&* Liddle, 2012).

In this study, we aimed to extend this previous work by investigating brain networks involved in the assignment of proximal salience in schizophrenia using the RM task in both fMRI and MEG. MEG is a very useful technique with excellent temporal resolution and fMRI can provide excellent spatial resolution and thus, these two techniques are complimentary and can be used together to obtain a better understanding of network function. In particular, the data from this multimodal study will allow us to explicitly test the inefficient cerebral recruitment hypothesis in relation to SN dysfunction in schizophrenia in a novel manner. Using fMRI, we aimed to examine the whole-brain effects of task-relevance in order to identify brain areas affected by aberrant salience assignment in schizophrenia.

We hypothesized that patients with schizophrenia would show inefficient recruitment of brain networks during the processing of relevant and irrelevant stimuli which might manifest as increased BOLD signal in the SN, CEN and other task-positive areas along with abnormalities in the DMN. Additionally, based on previous results of the RM task (Liddle *et al*, 2016), and the role of the beta band in long-range processing, we predicted abnormalities in the beta response to task in the SN during the processing of relevant trials in patients. Although the relationship between the BOLD signal and beta oscillations is not entirely clear, a previous study by Mantini and colleagues investigated this issue using resting-state simultaneous EEG-fMRI. They reported that beta power is positively correlated with the BOLD signal in the DMN but negatively correlated with the BOLD signal in task positive areas (Mantini *et al*, 2007). Another study using electrocorticography has also reported similar findings (Ossandón *et al*, 2011). Based on this work and our prediction of increased BOLD signal in task-positive areas including the SN, we hypothesized that patients with schizophrenia would also demonstrate reduced beta response to task in the SN, as a marker of inefficient cerebral recruitment.

3.3. Methods

3.3.1. Participants

Details relating to volunteer recruitment and clinical and cognitive assessments for this dataset have been reported in detail in chapter 2, section 2.1. Briefly, following a diagnostic consensus meeting, patients with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder were recruited to the study using the following inclusion criteria: 1) currently in a stable phase of illness (defined as a change of no more than 10 points in their Social and Occupational Functioning Assessment Scale (SOFAS) score (DSM-IV (American Psychiatric Association, 1994) between evaluation 6 weeks preceding and immediately preceding study participation), 2) age range 18-55 years, 3) IQ above 70, 4) no lifetime history of substance dependence or harmful use in the past 6 months, 5) no history of significant head trauma or medical conditions likely to have appreciable neurological or psychiatric effects, 6) no contraindications for MRI safety assessed by a standardized safety screening questionnaire.

As most patients were medicated, the median Defined Daily Dose (DDD) (WHO-CCDSM, 2003) was calculated separately for antipsychotics, mood stabilizers including lithium and antidepressants and no patient had a change in any of these prescriptions six weeks prior to study participation. Healthy controls with the same inclusion criteria (2-6) were recruited to the study using poster advertisements and those with a family history of psychotic illness were excluded. The two groups were matched for age, gender and parental socio-economic status. Details of clinical and cognitive assessments have been

reported in chapter 2 and the issue relating to appropriate matching criteria has been discussed previously in chapter 2 (section 2.1). This study was approved by the National Research Ethics Committee. Informed consent was obtained from all participants and an inconvenience allowance was provided.

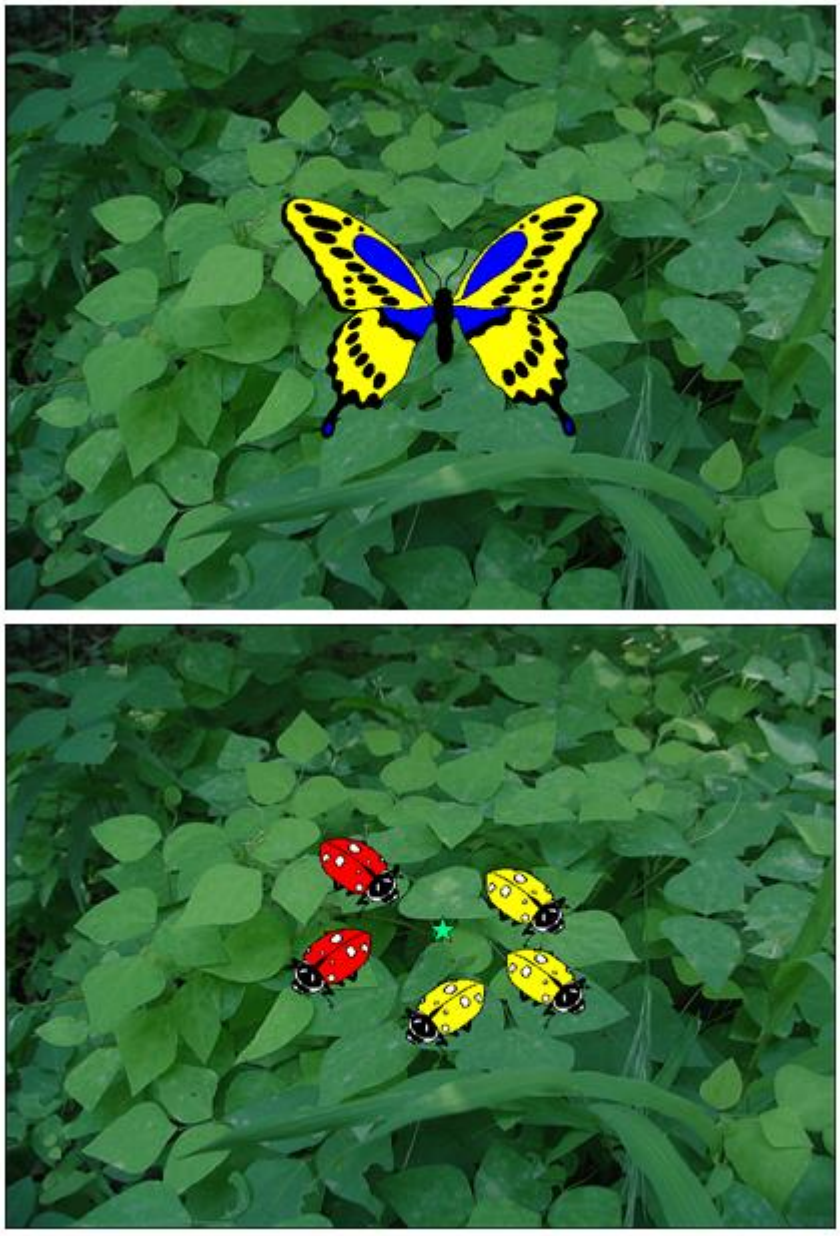
3.3.2. Relevance Modulation (RM) Task

This task has been described in detail previously in Liddle *et al* (2016) and also in Brookes *et al* (2012). Briefly, the RM task is a target-detection task with minimal motor response requirements and in which the task-relevance of the stimuli is manipulated such that some stimuli are relevant and others are irrelevant. Trials requiring a motor response i.e. target trials are rare, and discarded from subsequent analysis, however the relevant stimuli are attention-demanding due to the time it takes to identify whether or not a relevant stimulus type is a target. The irrelevant stimuli demand no attention. The relevant and irrelevant stimuli are presented in an alternating order and hence are predictable, in that the participant knows in advance whether the upcoming stimulus is a potential target (relevant) or will definitely not be a target (irrelevant). The task is designed such that the only consistent difference between relevant and irrelevant blocks is the task-relevance of the stimulus, and not its visual features or the specific cognitive task required in evaluating the stimulus. Thus, this task allows for a direct measurement of the phenomenon of proximal salience, as it can allow us to differentiate patterns of brain activity generated by relevant, salient stimuli and irrelevant stimuli, without motor response related confounds. The paradigm is described in detail below.

The same paradigm was used for both the fMRI and MEG scans. The task consisted of 8 blocks and two types of stimuli were used in each block: images of butterflies and ladybirds. Images of stimuli are provided in the Figure 1. Paired images of butterflies and ladybirds alternated regularly during each block i.e. two butterfly images followed by two ladybirds images (stimulus duration = 800 ms, mean inter-trial interval = 1310 ms, SD = 200 ms). Blocks lasted for 140s in total, including instructions, and consisted of 40 stimuli (20 butterfly images, 20 ladybird images), followed by a 30s rest period during which participants were asked to look at a fixation cross on the screen. At the beginning of each block, subjects were informed whether the relevant stimuli i.e. the stimuli to focus on were the butterflies or the ladybirds, and to ignore the intervening irrelevant stimuli. In blocks in which the butterflies were the relevant stimuli (BF blocks), participants were instructed to press a button when the butterfly on the screen matched an example butterfly shown at the start of the block based on three features – shape, inner wing colour and outer wing colour, while ignoring the interleaved images of ladybirds. In blocks in which ladybirds were relevant (LB blocks); participants were instructed to press the button if the number of red ladybirds in the image was equal to the number of yellow ladybirds, while ignoring the interleaved images of butterflies. In this study, the 8 blocks were presented in the in the following interleaved pattern: BF-LB-BF-LB-BF-LB-BF-LB. In line with previous studies (Brookes *et al*, 2012; Liddle *et al*, 2016), for both block types (BF and LB), the probability of a “target” image i.e. those to which the participant had to make a button press (e.g. a butterfly matching an example butterfly or equal number of red and yellow ladybirds) was 0.05. This low proportion of targets was

chosen in order to minimize motor responses while allowing participants to attend to the task and also loss of trials when analysing only trials without the confounding effect of an overt motor response.

Figure 1: Example stimuli from the relevance modulation task



3.3.3. Image acquisition

3.3.3.1. Functional MRI

The fMRI scans were acquired at the Sir Peter Mansfield Imaging Centre, University of Nottingham, using a 7 Tesla Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands). The multi-slice gradient echo-planar images (EPI) were acquired using a 32-channel head coil with SENSE factor 2.5, TE of 25 ms, flip angle 75°, 224 x 192 x 72 mm field of view. A 2 x 2 mm in-plane resolution was used with a slice thickness of 2 mm and a TR of 2000 ms. At each dynamic time-point a volume dataset was acquired consisting of 36 contiguous axial slices in ascending order. Data were acquired in 4 sessions and 142 functional images were acquired during each session. An anatomical T1 MPRAGE image (TE/TR=3.4/7.3ms) was acquired for each subject (1mm isotropic resolution, 256x256x180 matrix, flip angle 8°) for image registration.

3.3.3.2. MEG

MEG data were also acquired at the Sir Peter Mansfield Imaging Centre on the same day as the fMRI and anatomical scans. A 275 channel whole head CTF system (MEG International Services Ltd., Coquitlam, Canada) with a synthetic third order gradiometer configuration was used. 4 noisy sensors were switched off and in order to perform noise cancellation, 29 reference channels were also used. A 600Hz sampling rate and 150Hz low-pass anti-aliasing hardware filter were used in order to acquire the data. The location of the participant's head was measured at the start and end of data acquisition using three electromagnetic head position indicator coils placed at the nasion

and left and right pre-auricular points. These coils were then energized in order to acquire continuous head localization data within the MEG helmet as the subject was performing the task. A 3D digitizer (Polhemus, Isotrack) was used in order to map the surface shape of the subject's head relative to the three coils prior to data acquisition. The head surface for each participant was extracted from the 7T anatomical T1 MPRAGE image described in the previous section and the digital surface shape and coil locations were then registered to the head surface in order to allow for the spatial localization of MEG data.

3.3.4. Data processing

3.3.4.1. Behavioural Analysis

Task accuracy for both the fMRI and MEG paradigms were measured for each participant as a *d'* score (p-to-*z* transformed proportion of targets correctly identified minus *z*-transformed proportion of non-targets misidentified) (Green *&* Swets, 1966). 100% accurately identified targets were replaced with a value of 99.99% and 0% misidentified non-targets were replaced with a value of 0.01% in order to avoid an undefined z score. Independent sample t-tests were performed in order to compare differences between patients and controls in task accuracy and reaction times.

3.3.4.2. Functional MRI

1) Within-subject analyses

fMRI data were pre-processed in SPM8 using a standard pre-processing pipeline. Data from the 4 sessions were pre-processed independently. The functional scans were slice-time corrected and spatially realigned to the first image of the session. The scans were then spatially normalized to each subject's segmented, normalized structural image and then smoothed using a Gaussian kernel of 8mm full-width at half-maximum. The ArtRepair toolbox in SPM8 was used in order to correct for post-realignment residual errors using a nearest-neighbour interpolation approach (Mazaika *et al*, 2009).

Stimulus onsets were then modelled as blocks and block-types consisted of four types of trials – relevant butterflies, relevant ladybirds, irrelevant butterflies and irrelevant ladybirds. Blocks were convolved with a haemodynamic response function and a temporal derivative. Movement parameters were assessed for each participant using the Friston 24 approach (i.e. 6 head motion parameters, 6 head motion parameters one time point before, and the corresponding 12 squared values) (Friston *et al*, 1996) and nuisance regressors including the Friston 24 motion regressors, white matter and cerebrospinal fluid signal were included in the model. Data from all relevant blocks were collapsed together and similarly data from all irrelevant blocks were collapsed together and an all relevant – all irrelevant contrast was computed for each subject.

2) Group analyses

The all relevant – all irrelevant contrast images i.e. images representing within-subject differences at first level were entered into one sample t-tests for the entire sample with a voxel-wise threshold of Family-Wise Error (FWE) corrected $p < 0.05$ in order to examine the effects of proximal salience on the brain in the combined group. A stringent threshold was used in order to avoid making Type 1 errors in the identification of clusters activated by relevant vs irrelevant stimuli. Then, in order to test our main hypothesis of between-group mean differences in brain activations as a function of task-relevance, an independent sample t-test was computed using the all relevant – irrelevant first level contrast images with the *d'* score included as a covariate. A voxel-wise inclusion threshold of uncorrected $p < 0.05$ was used and significant clusters were then identified using a cluster-level threshold of FWE $p < 0.05$. Additionally, we also examined the effects of task performance on BOLD activations in the entire sample (voxel-wise inclusion threshold $p < 0.05$; cluster-level threshold FWE $p < 0.05$).

3.3.4.3. MEG

1) Pre-processing

The pre-processing pipeline for the MEG data has been described in detail previously in Liddle *et al*, 2016. MEG data were first visually examined for artefacts and trials containing excessive interference (e.g. interferences caused by the magnetomyogram) and such noisy trials were removed from that individual's dataset. Any trials in which the head was found to be more than 8mm (Euclidean distance) from the starting position were excluded using

continuous head localization information. Data from all target trials and all trials containing a button press were also removed, leaving only data from relevant and irrelevant trials without any button response. Finally, blocks in which more than 50% of trials were removed were discarded and only participants with 5 or more usable blocks were included in the analysis. In line with Liddle *et al*, 2016, an Independent Components Analysis (ICA)-derived insular network map from an independent group of healthy controls (Brookes *et al*, 2012) was used after weighting and thresholding in order to compare differences in SN function between healthy controls and schizophrenia patients.

The anatomical MPRAGE image for each subject was skull stripped and segmented using BET (Smith *et al*, 2002) and then downsampled spatially to an 8 mm isotropic resolution. The thresholded insular network map was then registered to each subject's downsampled anatomical space using FLIRT (Jenkinson *et al*, 2002). A multiple-local-sphere head model (Huang *et al*, 1999) and the dipole model (Sarvas, 1987) were used in order to compute lead fields for each subject and scalar beamformer weights and resultant time courses were derived at the centre of all downsampled voxels within the insular network map. A covariance matrix (1–150 Hz frequency window) was computed for a time window spanning the whole experiment in order to ensure measurement accuracy (Brookes *et al*, 2008) and then a weighted sum of electrophysiological time courses across the thresholded network was derived.

As described in Liddle *et al* (2016), the network time course was frequency filtered into 17 partially overlapping frequency bands of interest between 1 and 70 Hz (1–4, 2–6, 4–8, 6–10, 8–13, 10–15, 13–20, 15–25, 20–30, 25–35, 30–40, 45–50, 50–60, 55–65, 60–70). The Hilbert transform was used in order to compute the amplitude envelope for each band and envelope data from individual bands were concatenated to produce time–frequency (TF) spectrograms which were then averaged across all relevant and all irrelevant trials, respectively. Similar to the fMRI data, MEG data from relevant trials in butterfly-relevant and ladybird-relevant blocks were collapsed together, as were data from irrelevant trials.

For each frequency band, the mean oscillatory amplitude was computed during the 30s resting period of the paradigm at the end of each block of trials and these were then subtracted from the trial-averaged TF-spectrograms in order to measure deviation from each participant's own resting baseline values, resulting in time–frequency difference (TFD) spectrograms for each condition (relevant and irrelevant). These spectrograms were then averaged across participants in the control and the patient groups.

2) Statistical analyses

All statistical analyses were performed using SPSS 22 (IBM) and only data from participants included in the fMRI analyses were analysed in order to be able to relate findings from the two modalities. Only data from the first 1500 ms following stimulus presentation in each spectrogram were included in the analysis (900 samples) in order to avoid overlaps with the following trial. Mean beta amplitudes were computed for the two conditions and the two groups

and differences in these within and between groups were examined. In order to examine the modulation of beta band activity over time as a function of task relevance, data from the spectrograms were downsampled into 18 50-sample time bins for each participant for three beta bands (13–20, 15–25, and 20–30 Hz) and then averaged across the three bands. Firstly, in order to examine whether the overall pattern of increased ERD followed by ERS in the beta band for relevant trials compared to irrelevant trials was present in this dataset, these mean beta band time bin data for the entire sample were then entered into a repeated-measures ANOVA, with two within-subjects factors (relevance: relevant and irrelevant; time: 18 time-bins), but without any between-subjects factors. In line with Liddle *et al* (2016), the strength of the ERD–ERS pattern was operationalized as the extent to which the time series could be fitted by a cubic polynomial with a negative cubic term (to fit the ERD) and a positive quadratic term (to fit the ERS). If the results revealed a statistically significant time x relevance interaction, follow-up tests were conducted in order to further examine the cubic and quadratic terms and to determine if these were in with line with the hypothesis.

Subsequently, in order to test the main hypothesis regarding inefficient recruitment of the SN in schizophrenia, the repeated-measures ANOVA was performed again using with the same within-subjects factors and diagnosis as a between-subjects factor.

3.4. Results

3.4.1. Participants

39 healthy controls and 22 patients with schizophrenia completed RM task fMRI data acquisition. However, data from 1 healthy control was excluded due to errors with scanner triggers and data from 4 schizophrenia patients were also excluded from the analysis due to excessive movement. Thus, data from 38 healthy controls and 18 patients with schizophrenia were included in the fMRI analysis.

In the MEG RM task, 43 healthy controls and 38 patients with schizophrenia completed data acquisition. However, data from 5 healthy controls were excluded due to excessive movement and/or noise and data from 1 control was not included due to lack of anatomical MRI data for co-registration. Similarly, data from 9 patients were not included due to excessive movement and/or noise and data from 6 patients were not included due to lack of anatomical MRI data. Therefore, we had useable MEG data from 37 healthy controls and 23 schizophrenia patients.

Due to missing data, we did not have a complete overlap in the available fMRI and MEG data, and data from 32 healthy controls and 15 schizophrenia patients were included in the MEG analysis.

Clinical and demographic features of the sample are reported in Table 1.

Table 1: Clinical and demographic features of the sample

Features	fMRI Schizophrenia Patients (N = 18)	fMRI Healthy Controls (N = 38)	MEG Schizophrenia Patients (N = 18)	MEG Healthy Controls (N = 32)
	Mean/N (SD)			
Gender (Male/Female)	14/4	25/13	11/4	20/12
Age	26.44 (6.36)	27.07 (6.23)	26.13 (5.24)	26.90 (5.65)
Parental NS-SEC	2.67 (1.87)	1.79 (1.31)	2.53 (1.88)	1.90 (1.39)
Mean Illness Duration	51.72 (46.70)	-	51.93 (43.80)	-
DDD Antipsychotics	1.22 (0.77)	-	1.31 (0.79)	-
DDD Mood Stabilizers	0.02 (0.11)	-	0.03 (0.12)	-
DDD Antidepressants	0.40 (0.69)	-	0.48 (0.74)	-
SOFAS Score^{+}*	58.50 (16.87)	89.07 (4.70)	57.53 (18.20)	88.87 (4.79)
Quick IQ^{+}*	98.77 (10.02)	105.52 (8.33)	99.26 (10.68)	105.37 (8.27)
Anxiety/Depression^{+}*	1.61 (1.14)	0.81 (0.89)	1.60 (1.24)	0.78 (0.90)
Reality Distortion^{+}*	2.16 (2.50)	0.15 (0.59)	2.13 (2.47)	0.18 (0.64)
Psychomotor Poverty^{+}*	2.22 (2.01)	0.26 (0.50)	2.46 (2.09)	0.28 (0.52)
Disorganization	0.44 (0.61)	0.15 (0.36)	0.33 (0.48)	0.15 (0.36)
Psychomotor Excitation	0.66 (1.18)	0.36 (0.75)	0.73 (1.27)	0.40 (0.79)

NS-SEC: National Statistics Socio-Economic Classification; Symptom scores computed from SSPI: Signs and Symptoms of Psychotic Illness scale; DDD: Defined Daily Dose; SOFAS: Social and Occupational Functioning Assessment Scale. Mean Illness Duration reported in months

+ = significant difference between healthy controls and schizophrenia patients in the fMRI dataset ($p < 0.05$)

* = significant difference between healthy controls and schizophrenia patients in the MEG dataset ($p < 0.05$)

3.4.2. Behavioural Analysis

In the fMRI RM task, healthy controls were significantly more accurate at performing the task compared to patients with schizophrenia (t (54) = 3.97, p < 0.005). The mean d' score was 5.77 (SD = 1.85), representing a percentage accuracy of 99.99% (range 99.99% - 100%) for healthy controls and 3.71 (SD = 1.70) representing a percentage accuracy of 99.98% (range 97.77% - 99.99%) for schizophrenia patients. The mean RT was 820 ms (SD = 110) for healthy controls and for 790 ms (SD = 120) schizophrenia patients. There were no significant differences in RT in the fMRI task between the two groups.

Similarly, in the MEG RM task, healthy controls were again significantly more accurate at performing the task compared to schizophrenia patients (t (45) = 2.28, p < 0.05). The mean d' score was 5.02 (SD = 2.22), representing a percentage accuracy of 99.99% (range 99.74% - 100%) for healthy controls and 3.52 (SD = 1.78) representing a percentage accuracy of 99.97% (range 95.90% - 99.99%) for schizophrenia patients. There were no significant differences in RT in between the two groups with a mean RT of 765 ms (SD = 172) for healthy controls and for 778 ms (SD = 135) schizophrenia patients.

3.4.3. Functional MRI

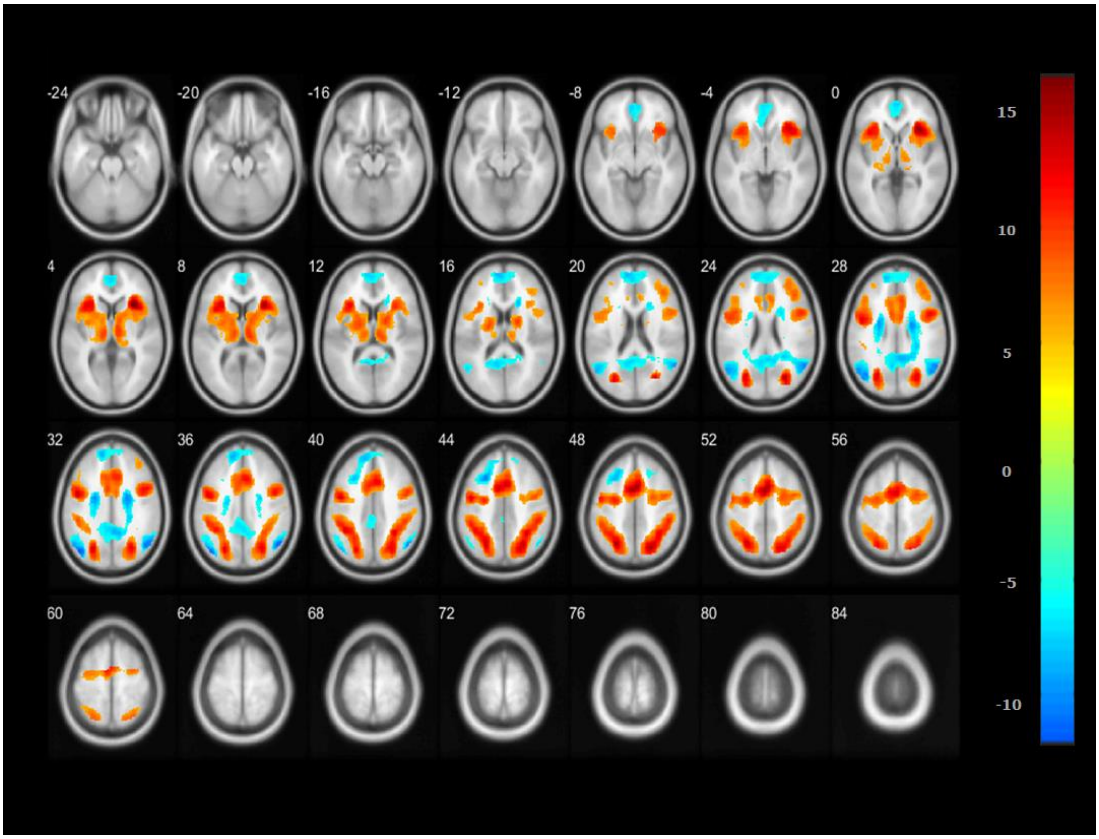
Results from the second level one sample t-test for the all relevant – all irrelevant contrast (representing within-subject differences across the entire sample) revealed significant activations in areas of the SN such as the bilateral AI and dACC along with areas of the CEN such as the bilateral dlPFC, bilateral inter-parietal sulcus among others, indicating the effect of

relevant stimuli on these networks (FWE $p < 0.005$) (Table 2, Figure 2). The peak with the highest t value was located on the right AI (MNI co-ordinates: 32 20 4). Additionally, significant suppression was also observed in DMN areas such as the PCC/precuneus, mPFC and the bilateral angular gyrus FWE $p < 0.005$) (Table 2, Figure 2).

The independent samples t-test results examining between-group differences between schizophrenia patients and healthy controls demonstrated significant differences between the two groups in the processing of relevant vs. irrelevant stimuli, after controlling for differences in task performance. Schizophrenia patients showed significantly increased BOLD activation in the right inter-parietal sulcus (FWE $p < 0.05$) compared to healthy controls. Patients also showed significantly decreased BOLD activation i.e. increased suppression in the right angular gyrus, PCC and mPFC (FWE $p < 0.05$). At a more lenient cluster-level threshold, patients also showed increased suppression in the left angular gyrus compared to healthy controls (FDR) $p < 0.05$). These results are presented in Table 3 and Figure 3.

Additionally, we also inspected the effect of task performance on BOLD activations in the entire sample. We found that improved task performance was associated with greater suppression in clusters including the mPFC, left angular gyrus and the PCC (FWE $p < 0.05$), and increased activation in the right inter-parietal sulcus (FWE $p < 0.005$) (Figure 4).

Figure 2: Activations and de-activations for the relevant – irrelevant condition in the entire sample (healthy controls and schizophrenia patients)*



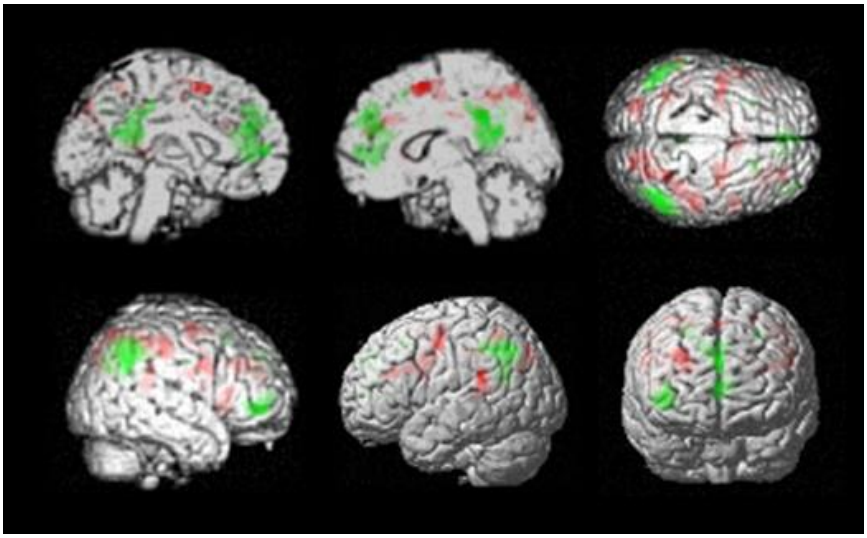
* Figure generated using xjview (Cui et al, 2011) One sample t-test, Voxelwise inclusion threshold = FWE $p < 0.05$. Red-yellow = relevant > irrelevant, Blue-green = irrelevant > relevant. Relevant stimuli produced significant activations in areas of the Salience Network such as the bilateral anterior insula and dorsal anterior cingulate cortex along with areas of the Central Executive Network such as the bilateral dorsolateral prefrontal cortex, bilateral inter-parietal sulcus etc. compared to irrelevant stimuli across the whole sample of participants (patients with schizophrenia and healthy controls (FWE $p < 0.005$). Relevant stimuli also produced a significant suppression in Default Mode Network areas such as the posterior cingulate cortex/precuneus, medial prefrontal cortex and the bilateral angular gyrus (FWE $p < 0.005$).

Table 2: One sample t-test results showing brain areas activated and de-activated by the entire sample (healthy controls and schizophrenia patients) in the relevant - irrelevant condition*

Regions	**Peak MNI co-ordinates**	**t value**	**Cluster size (voxels)**
Relevant > Irrelevant			
right insula	32 20 4	16.32	14151
right inter-parietal sulcus	28 -74 22	14.88	3474
left inter-parietal sulcus	-24 -62 50	13.99	3317
Irrelevant > Relevant			
left angular gyrus	-42 -72 32	11.92	1089
right angular gyrus	50 -64 36	11.62	797
middle frontal gyrus	-24 20 44	10.45	2730
cingulate gyrus	8 -48 30	9.25	1738

Voxel-wise inclusion threshold – FWE $p < 0.05$, cluster-level significance - FWE $p < 0.005$

Figure 3: Group differences between schizophrenia patients and healthy controls for the relevant - irrelevant condition displayed on render view*



* Voxelwise inclusion threshold = uncorrected $p < 0.05$, significant clusters identified cluster-level significance FWE $p < 0.05$, Figure generated using xjview (Cui et al, 2011). Red = schizophrenia patients > healthy controls, Green = controls > schizophrenia patients. Results after correcting for differences in task performance between the two groups: Patients with schizophrenia showed significantly increased BOLD activation in the right inter-parietal sulcus (FWE $p < 0.05$) compared to healthy controls (red). Patients also showed significantly decreased BOLD activation i.e. increased suppression in the right angular gyrus, posterior cingulate cortex and medial prefrontal cortex (FWE $p < 0.05$). At a more lenient cluster-level threshold, patients also showed increased suppression in the left angular gyrus compared to healthy controls (FDR $p < 0.05$) (green).

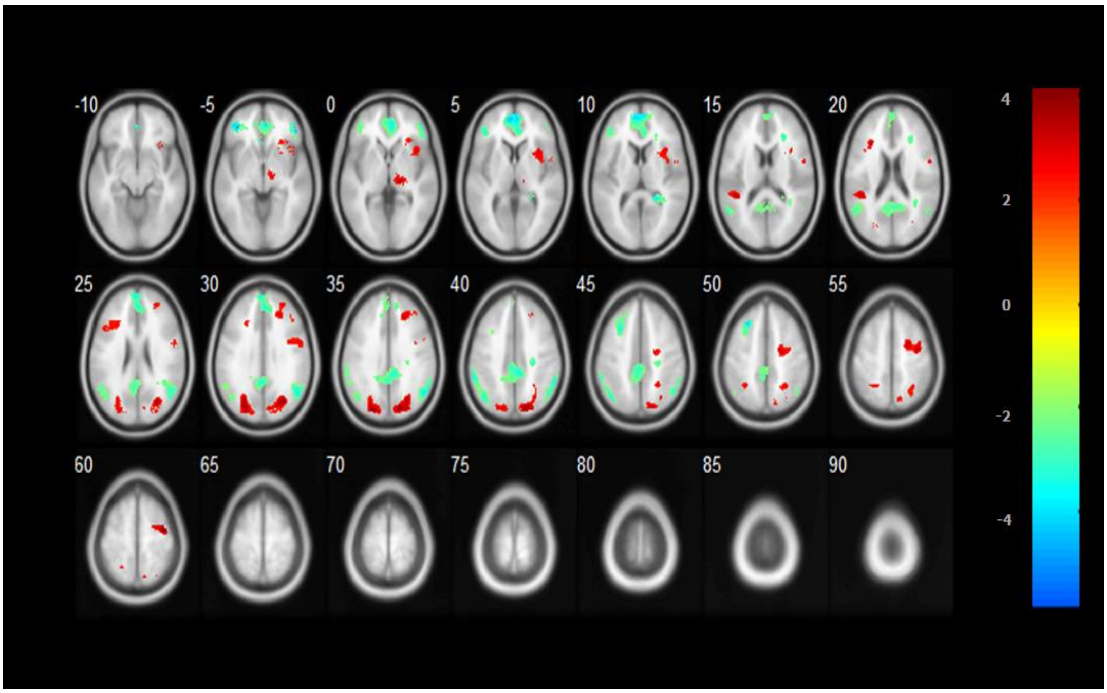
Table 3: Independent sample t-test results showing group differences between schizophrenia patients and healthy controls for the relevant – irrelevant condition

Regions	**Peak MNI co-ordinates**	**t value**	**Cluster size (voxels)**
Controls > Patients			
right angular gyrus	48 -60 38	4.65	970
left angular gyrus*	-54 -52 44	3.55	602
cingulate gyrus	12 -40 34	3.51	906
medial frontal gyrus	2 46 24	3.45	7.96
Patients > Controls			
right inter-parietal sulcus	-42 -40 46	3.74	1432

Voxel-wise inclusion threshold – uncorrected $p < 0.05$, cluster-level significance - FWE $p < 0.05$

* Cluster-level significance – FDR $p < 0.05$

Figure 4: Positive (red-yellow) and negative (blue-green) effects of task performance in relevant - irrelevant condition the entire sample (healthy controls and schizophrenia patients)*



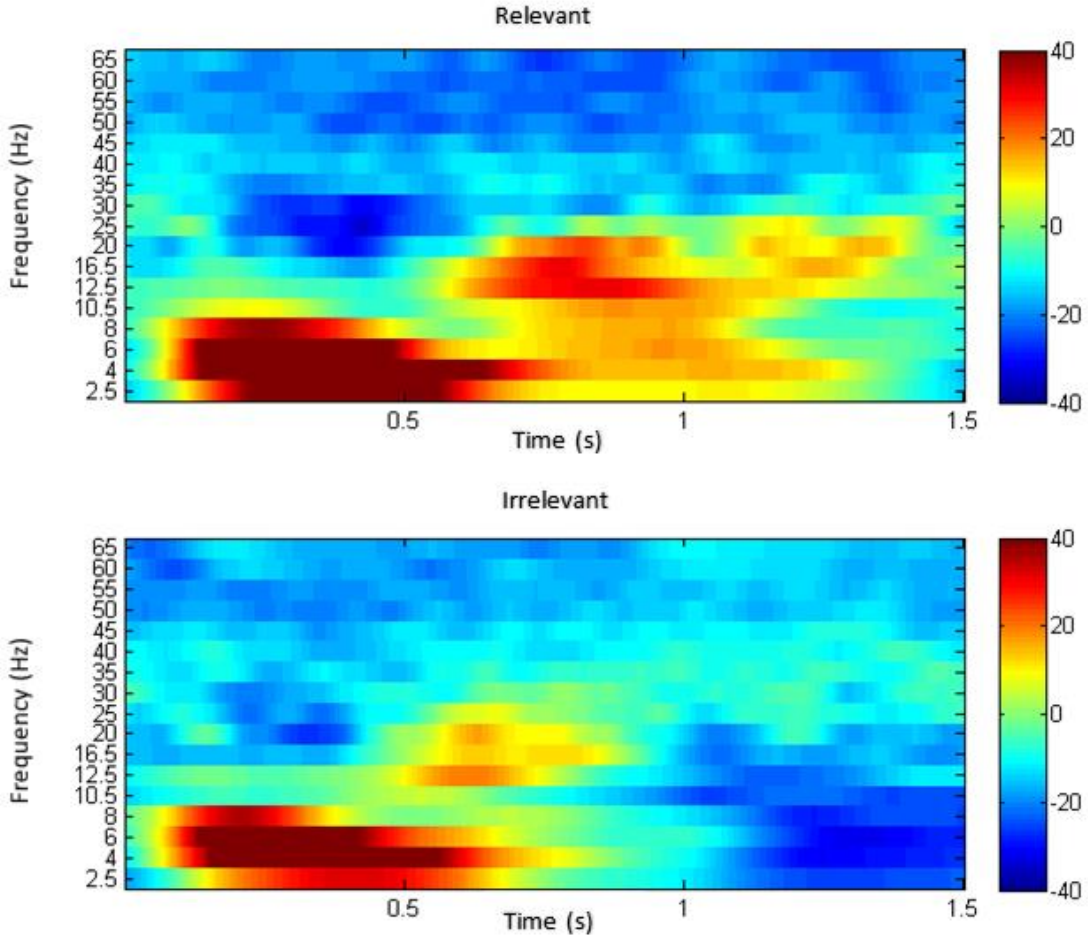
* Voxelwise inclusion threshold = uncorrected $p < 0.05$, significant clusters identified using cluster-level significance FWE $p < 0.05$, Figure generated using xjview (Cui et al, 2011). Improved task performance was associated with greater suppression in clusters including the mPFC, left angular gyrus and the PCC (FWE $p < 0.05$), and increased activation in the right inter-parietal sulcus (FWE $p < 0.005$) in the entire sample.

3.4.4. MEG

3.4.4.1. Time Frequency Difference Spectrograms

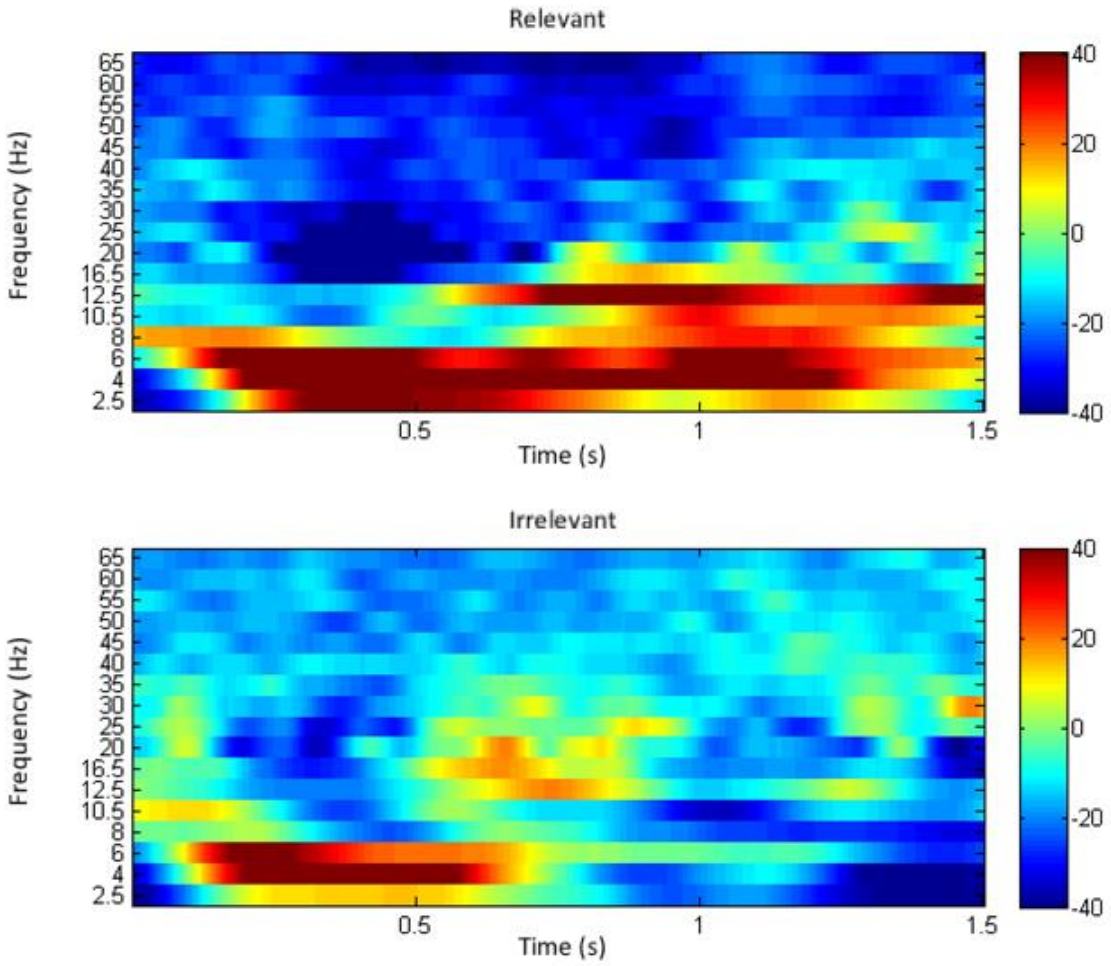
The insular network TFD spectrograms for the relevant and irrelevant conditions (representing a difference between beta amplitude during each condition relative to resting baseline) in both groups are presented in Figure 5A and 5B. Visual inspection of the TFD spectrograms revealed that these results are roughly in line with Liddle *et al*, 2016, although the differences observed in this study between the two groups were not as striking.

Figure 5A: Time frequency difference spectrograms for the relevant and irrelevant conditions in healthy controls



Time frequency difference spectrograms representing oscillatory activity (relative to resting baseline) for the first 1.5s following stimulus presentation in the relevant and irrelevant condition in healthy controls. During relevant trials, healthy controls show a brief period of event related desynchronization (ERD) in the beta band followed by a period of event related synchronization (ERS). These effects are not prominent in the irrelevant condition.

Figure 5B: Time frequency difference spectrograms for the relevant and irrelevant conditions in schizophrenia patients



Time frequency difference spectrograms representing oscillatory activity (relative to resting baseline) for the first 1.5s following stimulus presentation in the relevant and irrelevant condition in patients with schizophrenia. During relevant trials, patients demonstrate marked period of event related desynchronization (ERD) in the beta band; however this is not followed by period of event related synchronization (ERS). In the irrelevant condition, patients show attenuated beta ERD compared to the relevant condition, followed by beta ERS.

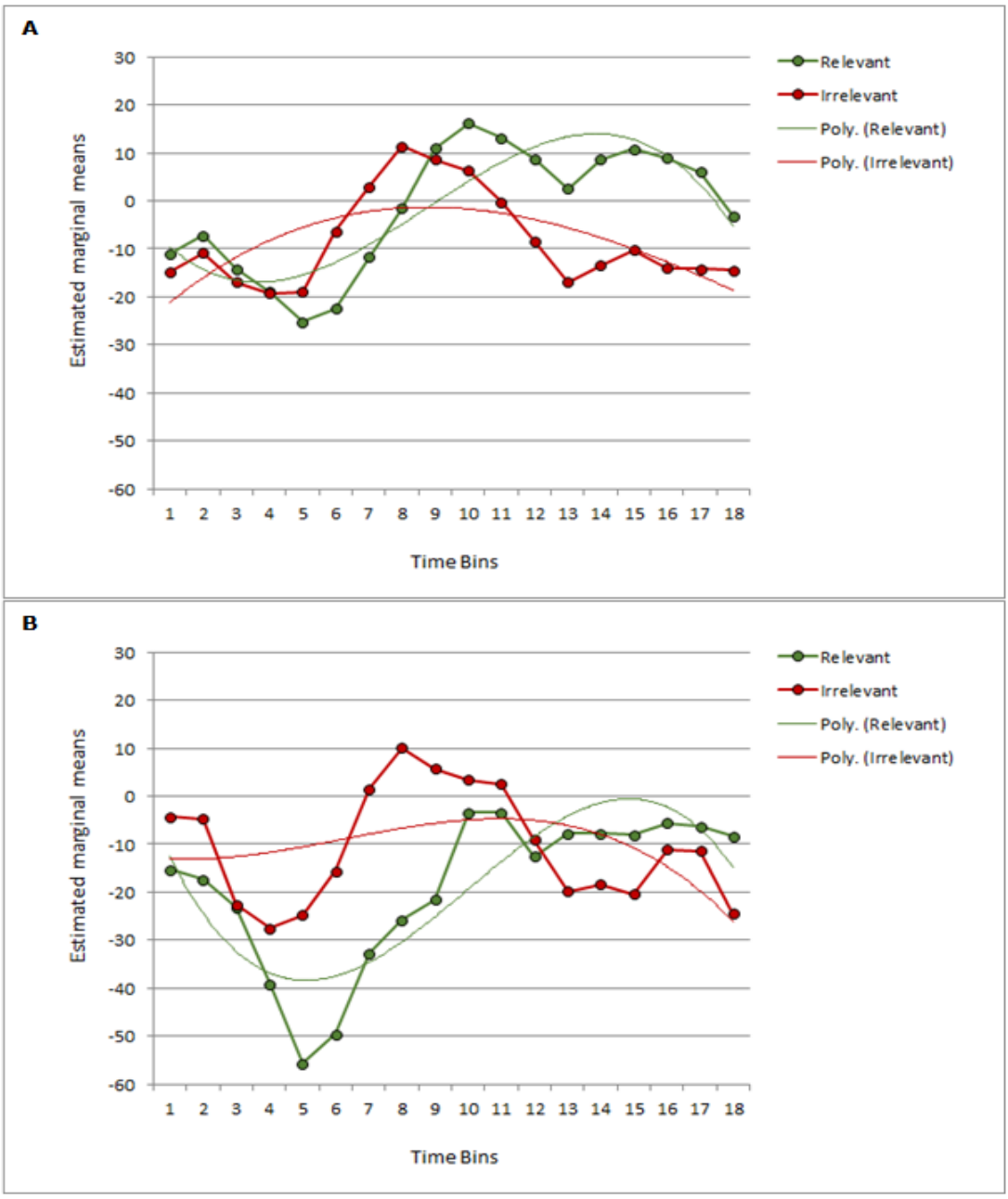
3.4.4.2. Statistical analyses

Results from the repeated measures ANOVA with a Greenhouse-Geisser correction computed in the entire sample revealed significant *F* values for the time (F (4.941, 227.27) = 16.650, $p < 0.001$) and time × relevance factors (F (3.967, 182.467) = 8.998, $p < 0.001$). Due to the significant time x relevance interaction, a 3rd order polynomial was fitted in order to examine the cubic and quadratic terms. As hypothesized, we found significantly negative cubic (to fit the ERD) ($p < 0.001$) and positive quadratic (to fit the ERS) ($p < 0.001$) terms. Upon further inspection of the relevant and irrelevant conditions separately, we found significantly negative cubic and positive quadratic terms for relevant condition ($p < 0.001$). However, the cubic and quadratic terms were not statistically significant for the irrelevant condition.

We then performed a second repeated measures ANOVA with diagnostic group as a between-subjects factor. We found a significant relevance x diagnosis interaction (F (1, 45) = 5.831, $p < 0.05$). Follow-up tests revealed that in healthy controls, there was a trend toward a significant difference between the two conditions (F (1, 31) = 3.850, $p = 0.059$) with a lower mean beta amplitude in the irrelevant condition (mean = -8.32, SD = 12.73) compared to the relevant condition (mean = -1.61, SD = 18.28). No difference between the two conditions was observed in patients with schizophrenia. We also found a significant difference between the two groups in the relevant condition (F (1, 45) = 5.361, $p < 0.05$) with lower mean beta amplitude in schizophrenia patients (mean = -19.09, SD = 33.63) compared to healthy controls (mean = -1.61, SD = 18.28). However, no significant differences were observed in the irrelevant condition ($F < 1$). In this study, we found no time x

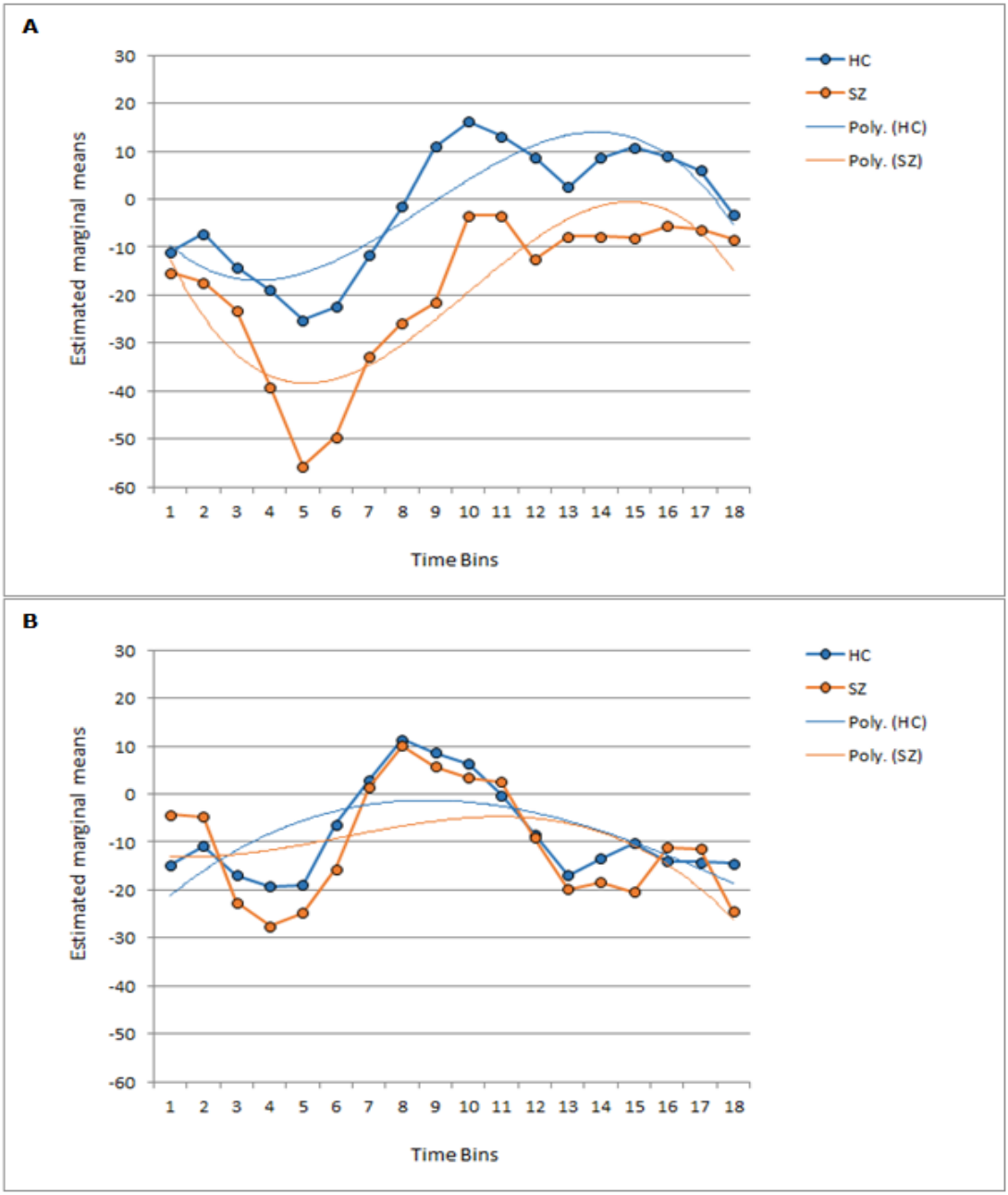
diagnosis interaction and additionally, the groups did not differ significantly in their modulation of beta over time by relevance (relevance × time × diagnosis interaction), unlike the results reported by Liddle *et al* (2016). Line plots representing changes in beta amplitude over the 18 time bins for the two conditions in the two groups are presented in Figure 6 and Figure 7. Mean beta amplitudes for the two conditions in the two groups are presented graphically in Figure 8.

Figure 6: Line plots representing changes in beta amplitude over the 18 time bins for the relevant and irrelevant conditions in healthy controls (panel A) and patients with schizophrenia (panel B).



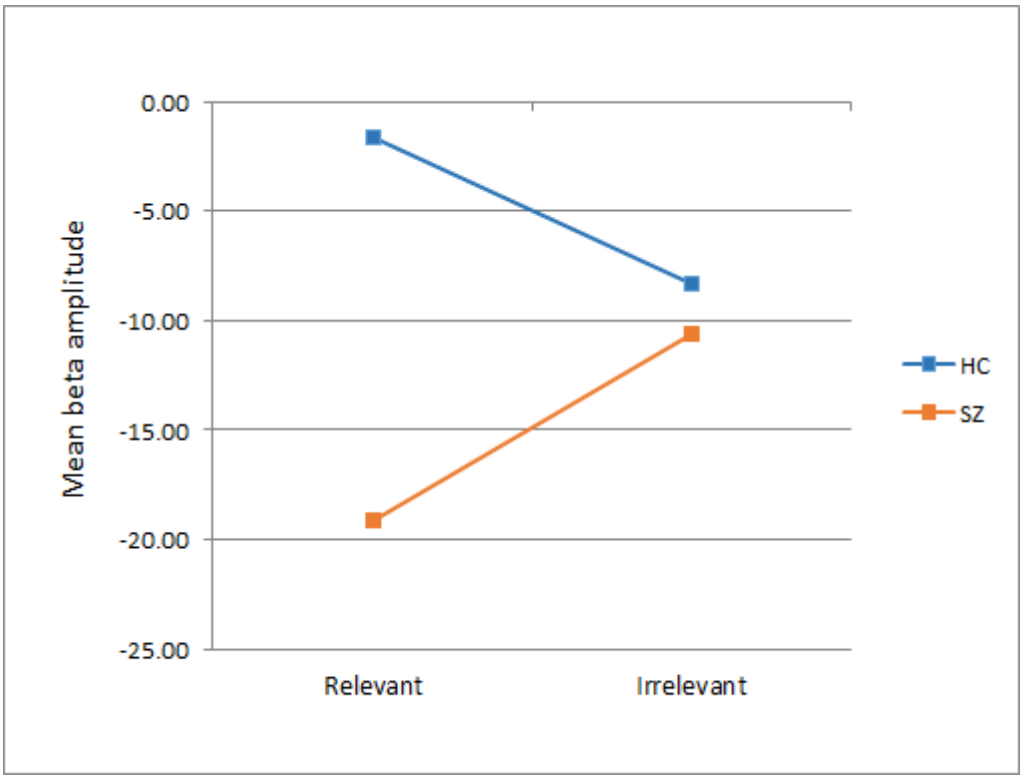
Healthy controls demonstrate greater event related desynchronization (ERD) and event related synchronization (ERS) in the beta band during relevant trials compared to irrelevant trials. Patients with schizophrenia demonstrate increased ERD during relevant trials compared to irrelevant trials, but increased ERS during irrelevant trials compared to relevant trials. *Trend lines represent cubic trend.

Figure 7: Line plots representing changes in beta amplitude over the 18 time bins in healthy controls (HC) and patients with schizophrenia (SZ) for the relevant (panel A) and irrelevant conditions (panel B).



Patients with schizophrenia demonstrate increased event related desynchronization (ERD) and decreased event related synchronization (ERS) in the beta band compared to healthy controls during relevant trials. No significant differences are observed between the two groups during irrelevant trials. Trend lines represent cubic trend.

Figure 8: Mean change in beta band amplitude from resting-baseline in the relevant and irrelevant conditions in healthy controls (HC) and schizophrenia patients (SZ)



Patients with schizophrenia demonstrate significantly decreased mean beta amplitudes in the relevant condition compared to healthy controls ($p < 0.05$). In the irrelevant condition, there are no significant differences in the mean beta amplitudes between the two groups.

3.5. Discussion

In this study, using both fMRI and MEG and a unique relevance modulation task, we aimed to test the link between the aberrant salience and inefficient cerebral recruitment hypotheses of schizophrenia. As expected, in the fMRI study, we found that the relevant condition resulted in significantly stronger activations in the SN (bilateral AI and dACC) and CEN regions (bilateral inter-parietal sulcus, dlPFC) and also other task positive areas such as the bilateral primary motor cortex compared to the irrelevant condition, in the entire sample. Additionally, relevant stimuli also produced significant suppression of DMN regions (PCC, mPFC and bilateral angular gyrus). In order to test the inefficient recruitment hypothesis, we then compared differences in activation patterns between schizophrenia patients and healthy controls after controlling for differences in task performance between the groups, with patients performing the task significantly poorly compared to controls. We found a greater effect of relevance i.e. a larger difference between the relevant and irrelevant conditions in patients compared to healthy controls, with significantly increased BOLD activation in CEN areas and significantly increased suppression in DMN regions in patients with schizophrenia. Moreover, upon inspecting the effects of task performance in the entire sample, we found that better task performance was associated with greater activity in CEN areas and increased suppression in DMN areas.

These results indicate that in order to achieve the same level of performance as healthy controls, patients with schizophrenia tend to over-engage the CEN and also over-suppress the DMN. Our results are in line with previous studies

reporting over-engagement of task positive brain areas in schizophrenia and the suggestion that patients need to recruit additional neuronal resources in order to perform the task as efficiently as healthy controls (Calicott *et al*, 2003; Potkin *et al*, 2009; Liddle *et al*, 2013; Jiang *et al*, 2015). It has been noted that inefficient recruitment in schizophrenia can be manifest as both hyper and hypo-activation of frontal areas, however, despite studies reporting both reduced and increased DMN suppression in schizophrenia (Harrison *et al*, 2007; Pomarol-Clotet *et al*, 2008; Whitfield-Gabrieli *et al*, 2009; Mannell *et al*, 2010; Anticevic *et al*, 2012; Fryer *et al*, 2013), inefficient recruitment in the context of DMN suppression in schizophrenia not been adequately addressed. Our results show that despite achieving lower performance compared to healthy controls, patients with schizophrenia also have a tendency to over-suppress the DMN. It is plausible that in order to effectively attend to the task and particularly to relevant stimuli, patients have to expend more effort to suppress conflicting internal thoughts and feelings and thus, DMN over-suppression in patients could be a compensatory mechanism. In another recent study which reported increased DMN suppression in schizophrenia patients, the authors similarly interpreted their findings as greater engagement or a more effortful processing mode in order to focus attention in space (Hahn *et al*, 2016).

In the second part of the study, we hypothesized that reduced neuronal efficiency in schizophrenia patients would manifest in the form of decreased beta response to task in the SN, examined using MEG. A previous MEG study of this task has reported abnormalities in the beta band in the SN in patients with schizophrenia (Liddle *et al*, 2016), in line with our hypothesis of inefficient

recruitment. Initial inspection of the TFD spectrograms (Figure 5A and 5B) and line plots (Figure 6 and 7) revealed that in healthy controls, relevant stimuli produced an initial reduction in beta amplitude (ERD) followed by an increase in beta amplitude (ERS) which reached or exceeded the baseline. In patients, the TFD spectrograms revealed a stronger ERD for relevant stimuli compared to healthy controls, but a weaker ERS, in line with our hypothesis of a reduced beta response. For the irrelevant stimuli, healthy controls and patients produced a weaker ERD compared to relevant stimuli and also a weaker ERS. However, no visually striking differences in the irrelevant condition were observed between the two groups.

The repeated measures ANOVA in the entire sample revealed findings in line with those reported by previous studies of the RM task (Brookes *et al*, 2012; Liddle *et al*, 2016) i.e. a significant time x relevance interaction with an increased ERD followed by increased ERS for relevant trials compared to irrelevant trials. Then, the same analysis was repeated with diagnosis as a between-subjects factor, in order to test our hypothesis of reduced beta response to task in the SN in patients. We found a significant relevance x diagnosis interaction, indicating that patients and controls differed in their modulation of beta response in the SN as a function of task relevance. Further analyses revealed that patients with schizophrenia demonstrated a significantly lower beta response to relevant trials compared to healthy controls, in line with our observation from the TFD spectrograms. However, there was no significant difference between the two groups in the irrelevant condition. These results suggest that patients suppress their beta response while attending to relevant stimuli and it is possible that this beta suppression

is also a compensatory mechanism. These findings are particularly intriguing in light of results from the fMRI analysis. In the fMRI data, increased DMN suppression was observed in subjects with better task performance. The fact that increased beta suppression during relevant trials was observed in schizophrenia patients who also demonstrated increased DMN suppression in fMRI lends further support to the idea that this might be a compensatory mechanism.

Overall, findings from both the fMRI and MEG study of the RM task support the notion that in order to attend effectively to relevant stimuli, patients with schizophrenia have to expend additional efforts to suppress attention to the internal world, resulting in inefficient recruitment of both task positive and negative brain areas. These results i.e. increased BOLD in task positive areas and decreased BOLD in DMN areas, along with reduced beta response to relevant stimuli in the SN are broadly consistent with the previously reported findings relating oscillatory activity to BOLD data (Mantini *et al*, 2007; Ossandón *et al*, 2011). However, certain limitations of this study need to be considered before making any final conclusions using this dataset. Firstly, these findings must be interpreted with extreme caution due to large between trial and subject variance in the data. Growing evidence suggests that the beta signal occurs in transient bursts and hence analysis of this signal should be done on a single trial basis (Sherman *et al*, 2016). Moreover, given the heterogeneity of the patient symptom profiles in this study, and data presented in the following chapter (chapter 4), it is likely that patients with different symptomatology and levels of functioning might display varying neural responses to salient stimuli. Therefore, further inspection and analyses

of this data are required before any firm conclusions can be drawn. These analyses are currently being undertaken but are beyond the scope of this thesis. Follow-up studies with larger sample sizes and more homogenous patient sub-groups will be required in order to fully investigate the effects of task relevance and proximal salience on the functioning of the SN in schizophrenia.

Chapter 4

Glutathione and glutamate in the Salience Network in schizophrenia

4.1. Abstract

Abnormalities of neural metabolite concentrations in schizophrenia may arise from cortical damage following neuroinflammatory processes implicated in acute episodes. Inflammation is associated with increased glutamate, whereas the antioxidant glutathione might provide defence against oxidative stress associated with inflammation. In this chapter, we aimed to investigate if patients with schizophrenia exhibit reduction in glutathione, glutamate and/or glutamine in the anterior cingulate cortex (ACC) and hypothesized that any reduction would be more prominent in patients with residual schizophrenia, consistent with a post-inflammatory response. This study included 28 patients with schizophrenia subdivided into “residual” and “non-residual” subgroups and 45 healthy participants matched for age, gender and parental socio-economic status. Glutathione, glutamate and glutamine concentrations in ACC, insula, and visual cortex measured using proton Magnetic Resonance Spectroscopy (MRS) at ultra-high field (7T), were used to differentiate between groups. We found significant correlations between glutathione and glutamate in all three voxels. Glutamine concentrations across the three voxels were significantly correlated with each other. Principal Components

Analysis (PCA) was therefore used to extract non-collinear predictor variables. Reduction in glutathione and glutamate in the ACC was a statistically significant differentiator of schizophrenia patients from healthy participants and also of patients with residual schizophrenia from both healthy controls and other schizophrenia patients (p <0.05). In light of other evidence indicating increased glutamate in early phase illness, these findings are consistent with the hypothesis that excito-toxicity during the acute phase of illness, possibly exacerbated by a pre-existing deficit in oxidative defences, might lead to reduced glutamate and glutathione in the residual phase.

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4.2. Introduction

Oxidative stress can result either from the excessive production of reactive oxygen species, for example due to inflammation, or from a reduction in oxidative defences, and can damage cell structures. Brain cells are particularly susceptible, owing to their low antioxidant defences and high oxidative metabolic activity. Glutathione, the brain's major intracellular antioxidant, thus has a vital role to play in maintaining brain cell health. Evidence for a role for oxidative stress in schizophrenia includes studies reporting reduced glutathione blood levels (Raffa *et al*, 2009) reduced post-mortem glutathione concentrations (Yao *et al*, 2006; Gawryluk *et al*, 2011) as well as genetic evidence implicating schizophrenia-related polymorphisms in genes involved in glutathione synthesis (Gysin *et al*, 2007). Other pathophysiological hypotheses of schizophrenia implicate abnormalities of glutamate, the brain's major excitatory neurotransmitter (Krystal *&* Moghaddam, 2011). One is that hypofunction of NMDA receptors (NMDAR) located on inhibitory GABA inter-neurons leads to reduced recruitment of these inter-neurons, resulting in an initial increase in pyramidal glutamatergic neurotransmission and positive symptoms, while a subsequent decrease in neurotransmission due to glutamate neurotoxicity results in negative symptoms (Gaspar *et al*, 2009; Plitman *et al*, 2014). Glutamatergic abnormalities in schizophrenia have been found in brain tissue studies (Hu *et al*, 2015); genetic association studies (Shi *et al*, 2008) and studies investigating the role of glutamatergic agonists to combat NMDAR hypofunction and reduce symptoms (Stone, 2011). There is also conflicting

evidence from studies using Magnetic Resonance Spectroscopy (MRS), discussed in greater detail below.

Evidence from studies of animals suggests a close relationship between glutamate and glutathione. Glutathione synthesis has been shown to be directly related to microglial glutamate uptake and the release of glutamate metabolites (Persson *et al*, 2006). In a review of the role of glutathione in protection against neuronal death, Bains *et al.* (Bains *et al*, 1997) present evidence that glutamatergic transmission activates biochemical pathways that generate free radicals and/or lower defence against damage by free radicals. Conversely, free radicals can increase the concentration of glutamate in the synaptic cleft by the release of glutamate or blockade of its re-uptake. In particular, oxidative stress reduces the uptake of glutamate in astrocytes, a key step in the recycling of glutamate (Volterra *et al*, 1994). This potentially creates a vicious cycle leading to damage by free radicals unless the glutamatergic transmission is associated with increase in glutathione.

Other evidence implicates NMDA receptors in the interaction between glutathione and glutamate. In a recent review article, Hardingham & Do presented evidence indicating that NMDAR hypofunction and oxidative stress may be reciprocally linked (Hardingham & Do, 2016). For instance, glutathione enhances NMDAR responses whereas its depletion results in NMDAR hypofunction (Steullet *et al*, 2006). Similarly, NMDAR hypofunction can also lead to oxidative damage (Papadia *et al*, 2008). Baxter *et al*. demonstrated that the glutathione system regulates antioxidant capacity via synaptic activity mediated by the NMDA receptors, and concluded that

glutathione deficits and NMDAR hypofunction occur in parallel (Baxter *et al*, 2015). These animal studies indicate that in an equilibrium state there is likely to be a close coupling between glutamate and glutathione. However it is plausible that if excessive stress were to cause a surge in glutamate that outstripped the capacity of glutathione to defend against the production of free radicals, neural tissue would be damaged leading to long term diminution of glutamatergic transmission and impaired brain function.

Magnetic resonance spectroscopy (MRS) is a non-invasive technique capable of measuring biochemical concentrations of glutathione, glutamate and other metabolites *in vivo*. Comparing schizophrenia patients with healthy participants, one MRS study found reduced glutathione in the medial prefrontal cortex (mPFC) in patients (Do *et al*, 2000), while another recent study reported reduced glutathione only in cases carrying a risk variant of the gene coding for the rate limiting enzyme in glutathione synthesis (Xin *et al*, 2016). Four other studies reported no significant differences in glutathione levels in the posterior medial frontal cortex (Monin *et al*, 2015), mPFC (Matsuzawa *et al,* 2008) or the anterior cingulate cortex (ACC) (Terpstra *et al*, 2005; Brandt *et al,* 2016), but two out of the four found a trend toward reduced glutathione in schizophrenia (Terpstra *et al*, 2005; Matsuzawa *et al,* 2008) and in one lowered levels of glutathione were associated with negative symptoms (Matsuzawa *et al,* 2008). In contrast, another study reported elevated levels of glutathione in the medial temporal lobes in first episode patients with schizophrenia (Wood *et al*, 2009b). However, note that this study used a lenient threshold for the inclusion of glutathione metabolite fits. A meta-analysis of oxidative stress markers in schizophrenia concluded that

oxidative stress levels might vary with clinical status (Flatow *et al*, 2013). These MRS studies also raise the possibility that the nature of glutathione abnormalities change with advancing phase of illness.

Because glutamate released during neurotransmission is recycled via glutamine in glial cells, glutamine may index glutamate neurotransmission. However, glutamate and glutamine each participate in several other distinct cell processes and hence may not be closely coupled (McKenna, 2007). MRS studies of glutamate and glutamine concentrations in schizophrenia have been inconsistent with some studies reporting an increase, others reporting a decrease or no abnormality (for reviews see Poels *et al*, 2014; Wijtenburg *et al*, 2015). One meta-analysis found that glutamate is reduced and glutamine is increased in the ACC in patients with schizophrenia, and both decrease more markedly with age in patients (Marsman *et al*, 2013). A more recent meta-analysis found higher medial-frontal Glx (glutamate + glutamine) levels in high-risk subjects (Merritt *et al*, 2016). Three recent studies have used ultra-high field strength (7T) MRS as its greater spectral resolution and signal-to-noise ratio enhances the ability to distinguish between abnormalities of these two metabolites. Two studies investigated the ACC/mPFC and one of these studies found increase glutamate/glutamine ratio (Rowland *et al*, 2016) while the other found no significant abnormalities in glutamate levels in schizophrenia (Marsman *et al*, 2014). A third study found reduced glutamate in the occipital cortex in schizophrenia patients compared to healthy controls (Thakkar *et al*, 2017).

As described above, most of the evidence for glutathione and glutamate/glutamine abnormalities in schizophrenia relate to the ACC, one of the key nodes of the SN. Results from several studies suggest that in the ACC at least, glutamatergic-related levels might be increased in the early phase of the illness or in unmedicated patients. In chronic patients, the levels of these neurochemicals may be reduced or unchanged depending on the persistence of symptoms or other factors associated with long-term illness (Marsman *et al*, 2013; Poels *et al*, 2014; Wijtenburg *et al*, 2015; Merritt *et al*, 2016). However, to our knowledge, glutathione and glutamate/glutamine levels in the insula have not been investigated to date in patients with schizophrenia. Given the important role played by the SN in co-ordinating the efficient recruitment of other brain networks and in the pathophysiology of schizophrenia (discussed in chapter 3), understanding the nature of neurochemical dysfunction in the SN would allow us to investigate the links between these abnormalities and SN activity and connectivity. To this effect, it is essential to investigate levels of glutathione, glutamate and glutamine in the ACC and the insula to determine if concentrations of these neurochemicals are affected only in the ACC or also in the insula in patients.

In light of this and the heterogeneity of prior findings, in this study, ultra-high field (7T) proton MRS (^{1}H-MRS) was used to investigate if during a stable phase of the illness, patients with schizophrenia exhibit decreased glutathione, glutamate and/or glutamine in the ACC and/or in the left insula. Furthermore, we hypothesized that any reduction would be more prominent in patients with residual schizophrenia, consistent with the hypothesis that decreases in glutamate and glutathione in the stable phase is a consequence

of damage arising in a preceding acute phase. “Residual schizophrenia” is defined in the ICD-10 as the chronic stage in the development of the illness, in which there has been a clear progression from an early stage with positive psychotic symptoms to a later stage characterized by long-term negative symptoms and impairments (which might include poor self-care and social performance), and a reduction in frequency and severity of positive symptoms such as hallucinations and delusions (World Health Organization, 1992). Although the DSM-IV (American Psychiatric Association, 1994) does not explicitly define residual schizophrenia, it describes as typical a course of the illness in which positive symptoms diminish while negative symptoms persist. If residual schizophrenia reflects neural damage due to oxidative stress, levels of glutathione, glutamate and glutamine might be lower in patients with residual schizophrenia than in other schizophrenia patients or healthy participants. Since animal studies have demonstrated a close link between glutathione and glutamate uptake into both microglia and astrocytes, and in NMDA receptor function, it was predicted that glutathione and glutamate levels in the brain will be correlated across all subjects.

4.3. Methods

4.3.1. Participants

Details relating to volunteer recruitment and clinical and cognitive assessments for this dataset have been reported in detail in chapter 2, section 2.1, and summarized in chapter 3, section 3.3.1.

As the DSM does not include explicit criteria for residual schizophrenia, ICD-10 criteria were used to operationalise residual schizophrenia and divide the patient sample into two subgroups: those with residual schizophrenia and those without residual schizophrenia, that we designated 'non-residual schizophrenia'. Operational criteria were as follows:

1) Cases of schizophrenia, currently in a stable phase of illness, defined by no change in SOFAS score of greater than 10 units in the preceding 6 weeks, who previously exhibited prominent delusions, hallucinations and or positive thought disorder, recorded in the case-file in an earlier phase of illness;

2) Currently exhibiting a combined score for delusions, hallucinations and positive thought disorder, assessed using the SSPI, no greater than 1 (a score of 1 indicates clinical features near the upper boundary of normal, and are questionably abnormal);

3) Currently exhibit a combined score for negative symptoms (poverty of speech, flat affect, decreased spontaneous movement and anhedonia) of 2 or greater, and/or a score for occupational and social function less than 70 assessed using the SOFAS scale.

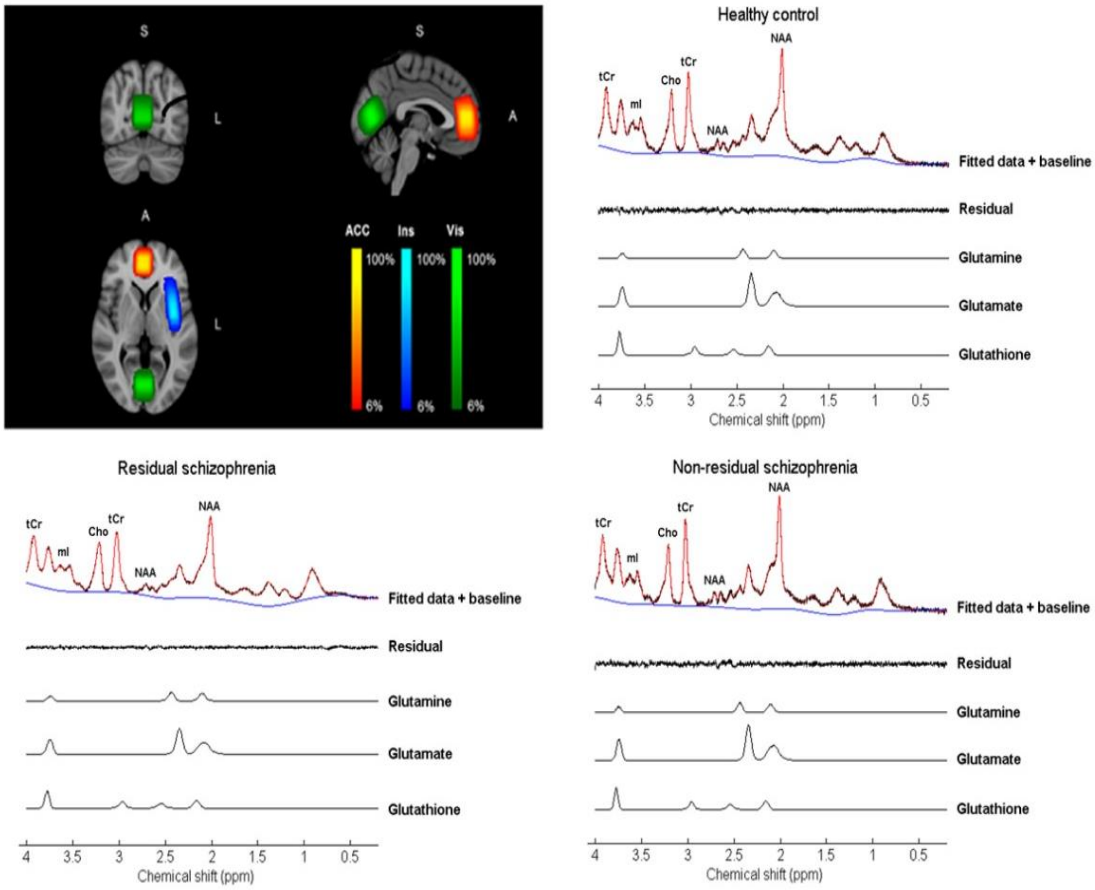
It should be noted that the group of cases classified as non-residual cases in this study were expected to be heterogeneous, potentially including cases with substantial persisting positive symptoms, cases with no appreciable persisting negative symptoms and cases with no history of a florid episode of illness. This study was approved by the National Research Ethics Committee. All participants gave informed consent and received an inconvenience allowance.

4.3.2. Image acquisition

All scans were conducted at the Sir Peter Mansfield Imaging Centre, University of Nottingham, using a 7 Tesla Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands), a volume transmit head coil, and a 32-channel receive head coil. MRS data were collected using a ^{1}H-MRS single voxel short TE STEAM (STimulated Echo Acquisition Mode) sequence (TE/TM/TR=17/17/2000ms) with 8 phase cycle steps, 4096 samples and a 4kHz bandwidth. 288 spectra were collected using the Multiply Optimised Insensitive Suppression Train (MOIST) technique for water suppression. Two spectra were collected without water suppression in order to correct for absolute concentrations using water referencing. A B_0 field map was acquired and parcellated shimming was used to enhance B_0 homogeneity (Poole *&* Bowtell, 2008). As the main aim of the study was to investigate neurochemical abnormalities in the SN, the primary voxels of interest (VOI) were placed in the ACC (20x18x25mm^3) and the left insula (40x12x18mm^3), two key nodes of the salience network which are hypothesized to play a key role in schizophrenia (Palaniyappan *&* Liddle, 2012). A comparison VOI

(20x22x20mm^3) was placed in the visual cortex. See Figure 9 for a sample spectrum and voxel placements. As described previously in chapter 3, an anatomical T1 MPRAGE image (TE/TR=3.4/7.3ms) was acquired for each subject (1mm isotropic resolution, 256x256x180 matrix, flip angle 8°) in order to aid VOI placement and for co-registration.

Figure 9: Average voxel placements and illustrative 1H spectra



Average voxel placements (ACC – anterior cingulate cortex, Ins – left insula, Vis – visual cortex) across all subjects overlaid on an MNI brain; Illustrative 1H spectra with baseline and residuals from a voxel located in the ACC of subjects from all three groups (healthy controls, residual schizophrenia and non-residual schizophrenia) and corresponding fits from LCModel for glutamine (Gln), glutamate (Glu) and glutathione (GSH).

4.3.3. Data processing

The anatomical image was segmented into grey matter, white matter and cerebrospinal fluid (CSF) using SPM8 (Ashburner *et al,* 2012). Tissue volume fractions were calculated using the segmented images and spatial co-ordinates of voxels. MRS data acquired independently from the 32 channels were reformatted, phase-corrected and realigned to account for frequency drifts, and then combined using an optimised coil combination method for MRS (Hall *et al*, 2014). Metabolite concentrations were estimated by fitting predicted spectra to the observed spectra using LCmodel (Provencher, 1993) with a custom basis set which included 20 metabolites. Lipid resonances and macromolecules were also included during the LCModel fitting. Metabolite concentrations were normalised to the concentration of water calculated from the unsuppressed water spectra, as variations in the water content within the sample are expected to be much smaller than the variations in the metabolite level. The variance between subjects in water signal is typically in the range 1.5-2.5% (Neeb *et al*, 2008) whereas variance in other metabolites that might be used as reference is typically around 5%. We assessed the reliability of the model fitting using Cramér-Rao Lower Bound (CRLB) estimates of the precision of the quantification of concentrations for each metabolite. Data above a CRLB threshold of SD=20% were excluded from the analysis (Provencher, 2015). The calculated tissue fractions were used to correct metabolite concentrations for partial volume effects and relaxation attenuation in the voxel (Gasparovic *et al*, 2006). T1 and T2 water relaxation times in grey matter, white matter and CSF were taken from previous published reports (Rooney *et al*, 2007; Wright *et al*, 2008; Cox *et al*, 2010).

4.3.4. Statistical Analyses

Statistical analyses were performed using SPSS 22 (IBM). Based on predictions that glutathione and glutamate levels will be correlated across all subjects, bivariate Pearson correlations were examined between all metabolite measures. After adjusting metabolite concentrations to control for potentially confounding variables, Principal Component Analysis (PCA) was used to extract non-collinear predictors.

A hierarchical binary logistic regression was then employed to investigate the relationship between metabolite concentrations and diagnosis, controlling for age and gender. Bootstrapped, Bias-Corrected accelerated (BCa) *p* values for the parameter estimates were used (10,000 samples). We first sought to identify metabolite measures differentiating patients with schizophrenia from healthy participants, then measures that differentiated residual schizophrenia from non-residual schizophrenia; finally we checked for measures differentiating each schizophrenia subgroup from healthy participants. We also examined the correlations between metabolite component scores obtained from the PCA and age as well as antipsychotic DDD.

4.4. Results

4.4.1. Participants

From the 46 healthy participants and 41 patients with schizophrenia recruited for this study, 45 healthy participants, 13 patients with residual schizophrenia and 15 patients with non-residual schizophrenia were included in the analysis. Details of exclusions are as follows: 1 healthy control and 12 patients did not undergo MRS data acquisition due to discomfort or anxiety arising from lying inside the scanner. We did not acquire MRS data from the visual voxel for 1 patient who partially completed MRS acquisition. Bad spectral quality and errors during data acquisition led to loss of ACC MRS data for one patient, insula and visual MRS data for another and data from all three voxels for a third patient. Thus, for all three voxels, data from 45 healthy controls were entered into subsequent analyses. In the patient group, data from 27, 27 and 26 patients were entered into subsequent analyses for the ACC, left insula and visual voxels respectively. ACC glutamine data for 3 healthy controls, insula and visual glutamine data for 1 healthy control and insula glutamine data for 1 patient had to be excluded as they did not meet the CRLB criteria. Clinical and demographic features of the sample are reported in Table 4.

Table 4: Clinical and demographic features of the sample

Features	All Patients (N = 28)	Residual Schizophrenia (N = 13)	Non-Residual Schizophrenia (N = 15)	Controls (N = 45)
	Mean/N (SD)			
Gender (Male/Female)^x	20/8	6/7	14/1	29/16
Age	27.18 (6.54)	28.00 (6.46)	26.46 (6.75)	27.89 (7.54)
Parental NS-SEC	2.39 (1.72)	1.92 (1.49)	2.80 (1.85)	1.97 (1.43)
Mean Illness Duration	54.42 (48.43)	72.00 (56.02)	39.20 (36.03)	-
DDD Antipsychotics	1.21 (0.69)	1.37 (0.90)	1.06 (0.43)	-
DDD Mood Stabilizers	0.05 (0.22)	0.00	0.10 (0.30)	-
DDD Antidepressants	0.43 (0.91)	0.39 (0.66)	0.46 (1.10)	-
SOFAS Score*^	57.35 (16.25)	53.92 (17.00)	60.33 (15.52)	88.73 (5.82)
Quick IQ*^x	97.82 (9.66)	100 (10.13)	95.93 (9.16)	104 (9.69)
Anxiety/Depression*^x	1.57 (1.23)	1.31 (1.31)	1.80 (1.14)	0.78 (0.85)
Reality Distortion*^x^	2.11 (2.40)	0.38 (0.87)	3.60 (2.32)	0.13 (0.54)
Psychomotor Poverty*^+x^	2.29 (1.88)	1.62 (1.44)	2.87 (2.06)	0.24 (0.48)
Disorganization^+	0.46 (0.63)	0.62 (0.50)	0.33 (0.72)	0.20 (0.54)
Psychomotor Excitation	0.57 (1.03)	0.62 (1.12)	0.53 (0.99)	0.47 (0.84)

NS-SEC: National Statistics Socio-Economic Classification; Symptom scores computed from SSPI: Signs and Symptoms of Psychotic Illness scale; DDD: Defined Daily Dose; SOFAS: Social and Occupational Functioning Assessment Scale. Mean Illness Duration reported in months

* = significant difference between healthy controls and the combined patient group ($p < 0.05$)

+ = significant difference between healthy controls and residual schizophrenia patients ($p < 0.05$)

x = significant difference between healthy controls and non-residual schizophrenia patients ($p < 0.05$)

^ = significant difference between residual and non-residual schizophrenia patients ($p < 0.05$)

4.4.2. MRS data quality and LCModel results

There were no significant differences in grey and white matter tissue compositions in the three voxels between the groups. In terms of data quality, there were no significant differences in the signal-to-noise ratio (SNR) in the three voxels between the two groups. However, there was a significant difference in spectral line-width in the visual cortex between patients and controls. Tissue composition and MRS data quality measures are reported in Table 5 and 6 below. Glutathione, glutamate and glutamine concentrations in all groups in the three voxels (estimated by LCModel and corrected for tissue fraction) are given in Table 7.

Table 5: Grey matter and white matter composition in all groups

Region	Tissue Type	Tissue composition (Mean/SD)			
		All Patients	Residual Schizophrenia	Non-residual Schizophrenia	Healthy Controls
ACC	Grey matter	0.729 (0.055)	0.734 (0.058)	0.730 (0.057)	0.743 (0.067)
	White matter	0.099 (0.044)	0.108 (0.050)	0.088 (0.036)	0.111 (0.064)
Vis	Grey matter	0.627 (0.055)	0.634 (0.061)	0.628 (0.055)	0.637 (0.046)
	White matter	0.289 (0.056)	0.289 (0.064)	0.283 (0.054)	0.285 (0.051)
Ins	Grey matter	0.663 (0.053)	0.675 (0.042)	0.658 (0.059)	0.676 (0.051)
	White matter	0.189 (0.079)	0.190 (0.087)	0.188 (0.071)	0.226 (0.085)

ACC = Anterior Cingulate Cortex, Vis = visual cortex, Ins = left insula

Grey and white matter compositions reported as tissue fractions

Table 6: MRS data quality measures in all groups

Region	Spectral quality measure	(Mean/SD)			
		All Patients	Residual Schizophrenia	Non-residual Schizophrenia	Healthy Controls
ACC	Line-width	17.58 (4.01)	17.18 (2.15)	17.56 (4.97)	16.98 (4.20)
	SNR	32.40 (9.74)	31.58 (9.42)	32.53 (9.72)	33.76 (10.60)
Vis	Line-width*	15.16 (3.31)	16.68 (4.27)	14.20 (1.82)	13.11 (1.87)
	SNR	41.96 (10.09)	42.18 (9.35)	41.33 (10.73)	46.22 (8.21)
Ins	Line-width	16.33 (6.14)	16.63 (4.77)	15.85 (6.94)	14.61 (3.07)
	SNR	23.60 (10.98)	25.00 (11.01)	22.40 (10.42)	24.49 (8.00)

ACC = anterior cingulate cortex, Vis = visual cortex, Ins = left insula, SNR = signal to noise ratio, line width reported in Hz.

* = significant difference between healthy controls and all patients, healthy controls and residual schizophrenia and also between residual and non-residual schizophrenia (p<.05)

Table 7: Glutathione (GSH), glutamate (Glu) and glutamine (Gln) concentrations among the three groups – healthy controls, patients with residual schizophrenia and patients with non-residual schizophrenia in the three regions - anterior cingulate cortex (ACC), visual cortex (Vis) and left insula (Ins)

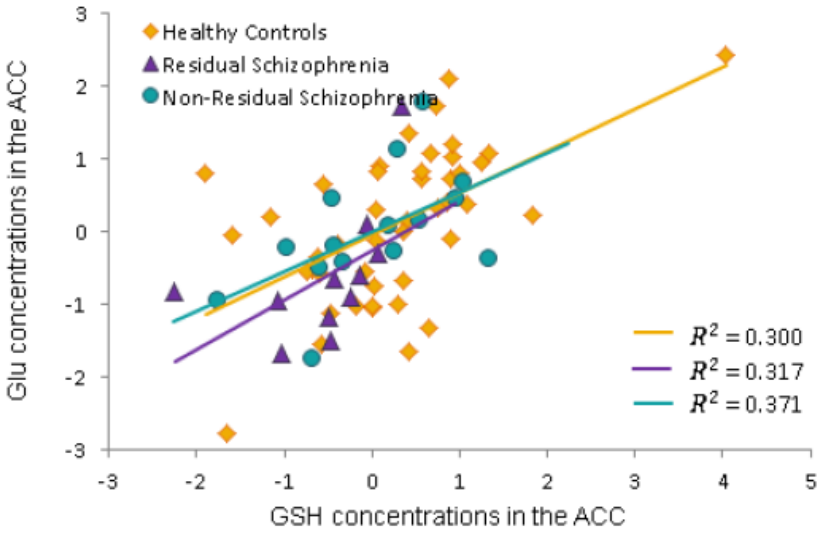
	Concentrations in millimolar (mM) (Mean/SD)*			
Region Metabolite	All Patients	Residual Schizophrenia	Non-residual Schizophrenia	Healthy Controls
ACC GSH	1.54 (0.26)	1.49 (0.23)	1.59 (0.28)	1.75 (0.31)
ACC Glu	6.00 (0.65)	5.67 (0.73)	6.27 (0.46)	6.21 (0.81)
ACC Gln	1.48 (0.35)	1.40 (0.29)	1.54 (0.39)	1.66 (0.31)
Vis GSH	1.46 (0.20)	1.45 (0.23)	1.47 (0.18)	1.50 (0.17)
Vis Glu	5.29 (0.50)	5.37 (0.55)	5.24 (0.48)	5.40 (0.50)
Vis Gln	1.22 (0.25)	1.18 (0.30)	1.25 (0.21)	1.29 (0.23)
Ins GSH	1.68 (0.25)	1.70 (0.24)	1.66 (0.27)	1.72 (0.19)
Ins Glu	6.39 (0.67)	6.55 (0.59)	6.26 (0.72)	6.44 (0.50)
Ins Gln	1.58 (0.30)	1.55 (0.23)	1.61 (0.35)	1.56 (0.27)

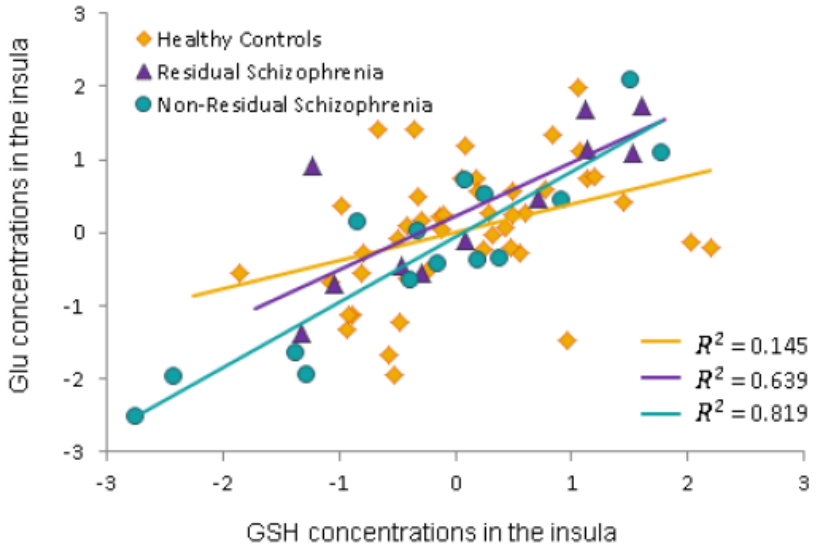
*Metabolite concentrations estimated by LCModel and corrected for tissue fraction

4.4.3. Correlations, confounds, and collinearity of predictors

Due to the significant difference in spectral line-width in the visual cortex between patients and controls, all visual cortex metabolites were regressed against visual line-width prior to further analyses. Since the non-residual schizophrenia group was unbalanced for gender, gender-adjusted values of all metabolites were used in all analyses for all groups. Examination of bivariate Pearson correlations between metabolites revealed strong correlations between glutamate and glutathione in all three voxels ($p < 0.05$) (Figure 10). In addition there were significant correlations ranging from 0.28 to 0.36 ($p < 0.05$) between glutamine levels in all three voxels, after adjusting for potential confounds as described above. We also inspected the correlation co-efficiency (cc) outputs from LCModel in order to ensure that any observed correlations between metabolite measures were not artefacts of LCModel fitting. The cc values for glutathione-glutamate, glutathione-glutamine and glutamate-glutamine in all three voxels in all groups were low and these have been reported in Table 8.

Figure 10: Correlation between glutathione (GSH) and glutamate (Glu) concentrations in the three regions (anterior cingulate cortex (ACC), visual cortex and insula) in the three groups (healthy controls, patients with residual schizophrenia and patients with non-residual schizophrenia). Values represent standardized residuals after regressing metabolite values on gender in the ACC and insula; and gender and spectral line-width in the visual cortex. Note: Correlation between GSH and Glu in the ACC in healthy controls remains significant even after removal of the subject with notably high GSH and Glu.


Healthy Controls
Residual Schizophrenia
Non-Residual Schizophrenia
$R^2 = 0.300$
$R^2 = 0.317$
$R^2 = 0.371$
Glu concentrations in the ACC
GSH concentrations in the ACC


Healthy Controls
Residual Schizophrenia
Non-Residual Schizophrenia
$R^2 = 0.145$
$R^2 = 0.639$
$R^2 = 0.819$
Glu concentrations in the insula
GSH concentrations in the insula

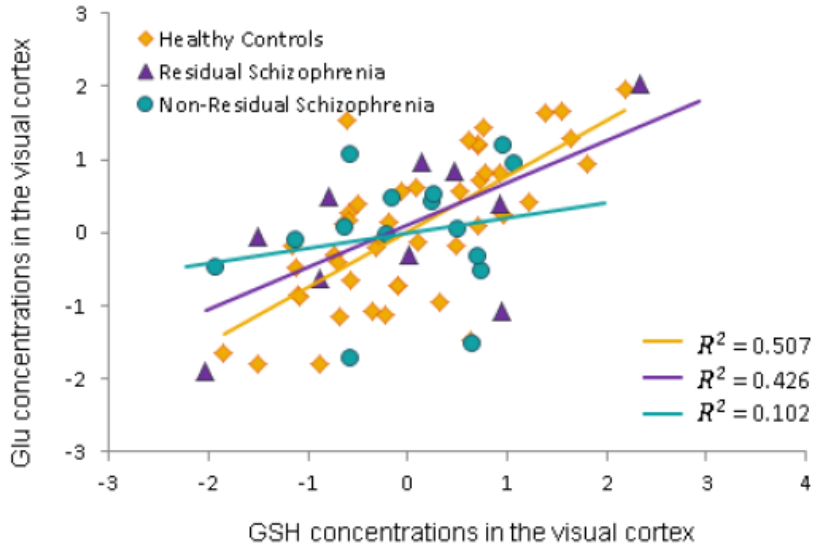

Healthy Controls
Residual Schizophrenia
Non-Residual Schizophrenia
$R^2 = 0.507$
$R^2 = 0.426$
$R^2 = 0.102$
Glu concentrations in the visual cortex
GSH concentrations in the visual cortex

Table 8: LCModel correlation co-efficiency (cc) values between metabolite fits in all groups

Region Metabolites	cc values: Mean (SD))			
	All Patients	Residual Schizophrenia	Non-residual Schizophrenia	Healthy Controls
ACC GSH-Glu	0.053 (0.096)	0.064 (0.086)	0.043 (0.105)	0.071 (0.112)
ACC GSH-Gln	-0.086 (0.125)	-0.069 (0.090)	-0.099 (0.149)	-0.029 (0.080)
ACC Glu-Gln	-0.037 (0.045)	-0.046 (0.045)	-0.030 (0.044)	-0.062 (0.050)
Vis GSH-Glu	-0.027 (0.087)	0.018 (0.099)	-0.061 (0.060)	-0.052 (0.051)
Vis GSH-Gln	-0.016 (0.070)	0.001 (0.061)	-0.028 (0.076)	-0.032 (0.078)
Vis Glu-Gln	-0.038 (0.046)	-0.054 (0.039)	-0.026 (0.048)	-0.021 (0.044)
Ins GSH-Glu	0.031 (0.129)	0.041 (0.131)	0.023 (0.132)	-0.001 (0.110)
Ins GSH-Gln	-0.120 (0.057)	-0.103 (0.063)	-0.132 (0.051)	-0.103 (0.078)
Ins Glu-Gln	-0.044 (0.047)	-0.044 (0.043)	-0.045 (0.051)	-0.045 (0.045)

ACC = anterior cingulate cortex; Vis = visual cortex; Ins = left insula; Gln = glutamine; Glu = glutamate, GSH = glutathione. Negative cc values between two metabolites indicate difficulty in resolving one metabolite from the other. Higher negative values = higher uncertainties with which two metabolites can be resolved from each other. Positive cc values between two metabolites indicate that the metabolites have fluctuations of the same sign, possibly due to fluctuations in the baseline (Provencher, 1993).

The pattern of correlations between metabolites in the patient and control groups examined separately was similar to that in the combined group. In particular, separate ANCOVAS performed for each voxel with glutamate as the dependent variable and with glutathione and diagnosis as predictors, demonstrated that glutathione was a strong predictor of glutamate in ACC and visual cortex (ACC: F (1,66) = 15.665, $p < 0.001$; Visual: F (1,65) = 26.912, $p < 0.001$), with no significant group by glutathione interaction, indicating no significant differences between groups in the prediction. In the insula, glutathione was a strong predictor of glutamate (F (1,66) = 50.085, $p < 0.001$) but there was an interaction arising from the stronger correlation between glutathione and glutamate in residual (r = 0.79) and non-residual schizophrenia (r = 0.90) than in healthy controls (r = 0.38). Nonetheless, the correlation was significant in all groups ($p < 0.05$).

Despite the fact that all three groups exhibited similar patterns of covariance, when identifying non-collinear predictors we nonetheless performed a weighted PCA with weights assigned to groups according to group size to avoid under-representation of the patient groups. Three components had eigenvalue > 1. Component loadings after varimax rotation are given in Table 9. The first component (accounting for 27.8% of the variance) loaded positively on glutamate and glutathione measures in the visual and insula voxels. The second component (18.5% of the variance) loaded positively on glutathione and glutamate in the ACC voxel and the third component (18.1% of the variance) loaded positively on glutamine measures in all three voxels. Separate PCA in patient and control groups identified three similar components with the same metabolite loadings exceeding 0.5 on

corresponding components in each group. There were no significant correlations between antipsychotic dose and metabolite component scores (visual-insula glutamate/glutathione component scores; ACC glutamate/glutathione component scores, glutamine component scores) in either patient group. Across the entire sample, there was a significant negative correlation between age and the ACC glutamate/glutathione component (r = -0.25, p < 0.05).

Table 9: Weighted Principal Components Analysis (PCA) loadings for glutamine, glutamate and glutathione in the whole sample

3 principal components explaining 64.38% of the variance (varimax rotation)			
	Component loadings		
Variables	Component 1	Component 2	Component 3
ACC glutamine	-0.048	0.378	0.730
Vis glutamine	0.061	-0.135	0.692
Ins glutamine	0.005	-0.027	0.757
ACC glutathione	0.137	0.861	0.088
Vis glutathione	0.721	0.006	0.056
Ins glutathione	0.737	0.282	0.131
ACC glutamate	0.189	0.801	-0.077
Vis glutamate	0.839	0.043	-0.082
Ins glutamate	0.820	0.198	-0.042

ACC = anterior cingulate cortex; Vis = visual cortex; Ins = left insula

4.4.4. Logistic Regression

For each logistic regression model, we entered age and gender in an initial block, then the three metabolite component scores in a second block.

4.4.4.1. Differentiating schizophrenia from healthy participants

Metabolite scores significantly differentiated patients with schizophrenia from healthy participants after controlling for age and gender, $\chi^2(3) = 11.393$, $p = 0.044$. Lower ACC glutamate/glutathione component scores, reflecting lower glutamate and glutathione levels in the ACC significantly raised the probability of schizophrenia (bootstrapped $p = 0.014$). Scores reflecting lower glutamine levels in all three voxels were associated with a trend towards raised probability of schizophrenia (bootstrapped $p = 0.096$).

4.4.4.2. Differentiating residual from non-residual schizophrenia

The metabolite component scores also differentiated patients with residual from non-residual schizophrenia after controlling for age and gender, $\chi^2(3) = 11.853$, $p = 0.037$. Lower ACC glutamate/glutathione scores were associated with elevated probability of residual schizophrenia (bootstrapped $p = 0.007$).

4.4.4.3. Differentiating each schizophrenia subgroup from healthy participants

Metabolite scores significantly differentiated between patients with residual schizophrenia from healthy participants, $\chi^2(3) = 12.840$, $p = 0.025$. Lower ACC glutamate/glutathione scores significantly increased the probability of residual schizophrenia relative to healthy control (bootstrapped $p = 0.003$).

The glutamine and visual-insula glutamate/glutathione components were not significant predictors. Metabolite scores did not significantly differentiate patients with non-residual schizophrenia from healthy participants.

4.5. Discussion

These results demonstrate that glutamate and glutathione are significantly correlated with each other in all three brain regions studied. This is consistent with the hypothesis of a mechanistic link between the antioxidant and glutamatergic systems in the human brain such that under steady state conditions, low levels of glutathione are associated with low levels of glutamatergic neurotransmission. The use of the unsuppressed water signal as a reference for the normalization of the metabolite signals might in principle introduce spurious correlations between the glutamate and glutathione signals. However, provided the variations in the water content within the sample are much smaller than the variations in the metabolite levels, the majority of the shared variance accounting for the observed correlations between normalised metabolite signals can be assumed to arise from shared variance in the metabolite concentrations (Neeb *et al*, 2008). While the observed correlations between glutamate and glutathione must be interpreted with some caution it is unlikely that the observed correlations between these metabolites can be accounted for by variation in the unsuppressed water signal. With regard to the comparisons between groups, it should be noted that there were no significant differences in grey and white matter tissue compositions in the three voxels between the groups (Table 5).

Furthermore, these results demonstrate that a factor representing variance shared between these metabolites in the ACC differentiates patients with schizophrenia from healthy controls, lower levels being associated with a diagnosis of schizophrenia. Additional analyses revealed that this factor also

differentiated patients with residual schizophrenia from healthy participants and also from patients with non-residual schizophrenia, lower levels being associated with residual schizophrenia in both contrasts. Thus, although reductions in glutamate-glutathione levels in the ACC were found in the combined patient group, as hypothesized these reductions were more prominent in patients with residual schizophrenia.

A trend towards lowered glutamine levels was also observed in patients in schizophrenia, irrespective of sub-group. Glutamate and glutamine are both implicated in glutamatergic neurotransmission, however they each participate in several other distinct cell processes and may not be closely coupled (McKenna, 2007). The finding that glutamine did not differentiate between residual and non-residual cases, suggests at most a partial overlap in the processes accounting for variance in glutamine and glutamate. The capacity of ultra-high field MRS to facilitate distinction between glutamate and glutamine revealed a distinguishable deficit in glutamate and glutathione, not substantially confounded by glutamine. We also observed a reduction in glutamate-glutathione levels in the ACC with age in accord with previous MRS findings (Marsman *et al*, 2013; Brandt *et al*, 2016) and consistent with the hypothesis that deceases in these metabolites can reflect degenerative changes.

Our finding that reduced glutamate and glutathione in the ACC in the stable phase of illness is most marked in those with a history of florid symptoms followed by residual negative symptoms and/or poor occupational and social function, is consistent with the hypothesis that florid illness might be

associated with damage leading to subsequent reductions in mental and neural activity mediated by glutamatergic neurotransmission. The evidence from animal studies that deficient defences against oxidative stress might promote damaging over-activity of glutamate (Bains *et al*, 1997; Persson *et al*, 2006) raises the possibly that in schizophrenia a long-standing deficit in glutathione increases the risk of excessive glutamatergic activity at times of stress. The observation that genetic glutathione deficits are related to schizophrenia supports this possibility in at least some cases (Gysin *et al*, 2007). Alternatively, even in the absence of a long-standing deficit of glutathione, a sufficiently severe stress might produce damaging glutamatergic hyperactivity, leading eventually to a residual deficit state. Irrespective of whether or not a glutathione deficit plays a primary role, these findings are consistent with the evidence that glutathione levels tend to be decreased in established schizophrenia (Do *et al*, 2000; Terpstra *et al*, 2005; Matsuzawa *et al*, 2008). Moreover, given that the ACC is a key node of the SN, these results suggest a complex interaction between abnormal neurochemical pathways, aberrant salience and symptomatology. It is plausible that reduced glutathione and glutamate levels in the SN could result in a lack of appropriate assignment of salience to relevant events and stimuli, leading to negative symptoms and poor socio-occupational functioning in the residual state. We did not find any significant differences in neurochemical levels in the left insula in this study. Due to significant correlations between metabolite concentrations, we used a PCA in order to extract non-collinear predictors and one component loaded strongly onto visual cortex and insula glutathione and glutamate levels. Hence, we were unable to investigate

differences in glutathione and glutamate in the insula independently. Given the strong correlations between glutathione and glutamate in the visual cortex and the insula, and no significant correlations between these neurochemical levels in the insula and the ACC, it is plausible that separate neurochemical processes govern the functioning of these two nodes of the salience network. Additionally, it is also possible that our choice of location of the ACC voxel has had an impact on this finding; given evidence from fMRI studies in which more dorsal ACC voxels are presented as part of the SN.

Nevertheless, our finding that shared variance in glutamate and glutathione in the ACC differentiates patients with schizophrenia from healthy controls is novel yet consistent with prior evidence indicating elevated glutamate early in the illness but diminished glutamate in older or more functionally impaired cases. This is in line with the hypothesis that excito-toxic damage during the acute phase can lead to a subsequent deficit state. This study has the advantage of high spectral resolution and signal-to-noise ratio of 7T MRS which facilitated good differentiation between glutamate and glutamine peaks.

However, several caveats also need to be considered. Firstly, although the sample size of the combined patient group was substantial; the sample sizes of the patient sub-groups were small and studies with larger sample sizes would be required in order to confirm findings related to residual schizophrenia. Moreover, the sample size of non-residual cases did not provide enough statistical power to test for differences between those with persisting positive symptoms and those without. Any follow-up studies of this work will benefit from the recruitment of a clinical group with acute psychotic

symptoms. Additionally, while healthy controls and the combined patient group were matched for gender, thereby avoiding influence of gender imbalance for our primary hypotheses, the non-residual group was unbalanced for gender. Although we controlled for this by using gender-adjusted metabolite concentrations and including gender as a variable in our logistic regression analyses, a gender-diagnosis interaction cannot be ruled out in comparisons involving the non-residual group. We investigated the relationship between current antipsychotic medication intake and metabolite concentrations; however possible confounding effects of long-term medication use need to be considered. Additionally, as mentioned previously, future studies should consider an alternative dorsal voxel placement for the ACC owing to evidence from fMRI studies, and in order to investigate whether neurochemical changes in the dorsal ACC are different to those observed in the medial prefrontal cortex. This study examined neurochemical levels only in the left insula. Follow-up studies which include both a left and right insula voxel are needed in order to fully examine neurochemical functioning in the SN in schizophrenia. Furthermore, longitudinal research, using 7T MRS to follow neurochemical changes across the various phases of illness, could elucidate whether neurochemical abnormalities in the early acute phase of schizophrenia differ from those seen in residual schizophrenia and whether these abnormalities are specific to the ACC or not.

Chapter 5

Aberrant causal connectivity within the Salience Network in schizophrenia

5.1. Abstract

Recent evidence suggests that the Salience Network (SN) is involved in co-ordinating the function of and switching between key brain networks such as the Central Executive Network (CEN) and the Default Mode Network (DMN). A number of research studies have now reported abnormalities in the causal connections between the SN and the CEN in patients with schizophrenia. Although it is clear that the SN dysfunction plays an important role in the pathophysiology of schizophrenia, few studies have investigated abnormalities in within-SN connectivity in schizophrenia. It is plausible that abnormal causal interactions within key SN nodes i.e. bilateral anterior insula (AI) and dorsal anterior cingulate cortex (dACC) could lead to failure in its co-ordinated recruitment of large scale brain networks. Hence, in this chapter, using resting-state fMRI and Granger Causality Analysis (GCA), we investigated whether there is aberrant causal or effective connectivity within the SN in schizophrenia. Additionally, we also examined whether any intra-SN causal dysfunction could be related to neurochemical abnormalities within the SN, which have been presented in chapter 4. 44 healthy controls and 25 patients with schizophrenia were included in the analysis. 7T resting-state

fMRI data were pre-processed and an ROI-wise GCA analysis was undertaken using pre-defined left AI, right AI and dACC masks. We found significant differences in effective connectivity between the left AI and dACC between the two groups ($p < 0.05$). Follow-up tests showed that in healthy controls, there were no significant causal influences between the left AI and the dACC. However, in schizophrenia patients the left AI exerted a significant positive causal influence on the dACC ($p < 0.01$) and the dACC exerted a significant negative causal influence on the left AI ($p < 0.05$). We also found a significant negative correlation between left AI to dACC causal influence and the ACC glutathione-glutamate component scores ($r = -0.50$, $p < 0.05$) and also a significant positive correlation between dACC to left AI causal influence and the ACC glutathione-glutamate component ($r = 0.43$, $p < 0.05$). Our results demonstrate aberrant processing within the SN in schizophrenia at rest, likely indicating an increased effort to suppress conflicting and disorganized internal thoughts. Furthermore, in light of the cardinal role of glutathione in cerebral defence against oxidative stress, the relationship with decreased glutathione raises the possibility of tissue damage due to inadequate defence against oxidative stress.

5.2. Introduction

The Salience Network (SN), anchored on the bilateral anterior insula (AI) and the dorsal anterior cingulate cortex (dACC), has generated a lot of interest in recent years due to its potential role in the neurophysiology of multiple psychiatric disorders including schizophrenia. As discussed previously in chapter 3, the SN plays an important role in detecting salient stimuli and events and subsequently enables further cognitive processing of these stimuli if required. Therefore, it controls the flow of bottom-up information received from sensory regions and only directs relevant information through to higher-order top-down information processing and decision making systems. In schizophrenia, one of the most robust findings reported by meta-analyses of structural imaging studies is grey matter abnormalities in the insula and the ACC, irrespective of differences in clinical features between various patient cohorts (e.g. Glahn *et al*, 2008; Fornito *et al*, 2009; Bora *et al*, 2011; Chan *et al*, 2011; Fusar-Poli *et al*, 2012). Growing evidence suggests that the SN plays a key role in co-ordinating the functioning of several key brain networks and other studies have now corroborated findings reported in 2008 by Sridharan and colleagues which demonstrated that the SN is involved in 'switching' between the Central Executive Network (CEN), the task positive network and the Default Mode Network (DMN), typically known to be a task negative network (Sridharan *et al*, 2008; Deshpande *et al*, 2011; Goulden *et al*, 2014; Chand *&* Dhamala, 2016). Therefore, research studies have investigated the question of aberrant causal connectivity between the SN and other brain regions and networks in the schizophrenia and have reported abnormalities in the causal interactions between the SN and the CEN and

also the DMN in patients (Palaniyappan *et al*, 2013; Moran *et al*, 2013; Manoliu *et al*, 2014). The potential role of the SN in the efficient recruitment of other brain regions has also been explored in chapter 3. Given this large body of evidence demonstrating the important role played by the SN in the recruitment of appropriate brain networks and abnormalities in this process in patients with schizophrenia, it is plausible that the integrity of SN itself potentially plays an important mechanistic role in controlling its interactions with other brain areas. Consequently, aberrant causal interactions within the key SN nodes i.e. bilateral AI and dACC could lead to failure in its co-ordinated recruitment of large-scale brain networks. Hence, this chapter will primarily explore the question of whether there is aberrant causal or 'effective' connectivity within the SN in schizophrenia. Moreover, this chapter will also aim to explore if any intra-SN causal dysfunction could be related to neurochemical abnormalities within the SN, which have been presented in chapter 4.

Few studies have reported abnormalities in within-SN connectivity in schizophrenia. For instance, using maximal time-lagged correlation analyses and Independent Components Analysis (ICA), White and colleagues have demonstrated reduced connectivity both within the SN and between the SN and DMN in patients with schizophrenia (White *et al*, 2010). Similarly, studies have reported reduced functional connectivity (Fc) within the SN in early stage paranoid schizophrenia (Pu *et al*, 2012) and in unmedicated first episode schizophrenia patients (Pang *et al*, 2017). Another recent study showed reduced intra-SN Fc and dynamic Fc in patients with schizophrenia at rest and in patients, and the aberrant dynamic Fc within the SN predicted Fc between

the SN and other networks. Additionally, these aberrant SN connectivity profiles were effective in differentiating schizophrenia patients from healthy controls. The authors concluded that abnormal patterns of connectivity between key brain networks in schizophrenia could be a result of unstable intra-SN connectivity and that SN dysfunction could be regarded as an intrinsic biomarker for schizophrenia (Wang *et al*, 2016b). These findings support the idea that aberrant interactions within the SN play an important role in how SN interacts with other brain regions. However, it must be noted that negative findings i.e. no differences in within SN connectivity in schizophrenia have also been reported (Woodward *et al*, 2011). The integrity of the SN has also been explored by studies using other imaging modalities. A recent study of cortical gyrification using graph theoretical measures demonstrated increased segregation in brain regions including the right AI in patients with schizophrenia compared to healthy controls, indicating that in patients, the insula has reduced influence on long-range brain networks but increased influence on neighbouring brain regions (Palaniyappan *et al*, 2015a). A study using functional MRI (fMRI) and Diffusion Tensor Imaging (DTI) in At Risk Mental State (ARMS) subjects reported that disrupted Fc between SN regions was related to nearby white matter tract abnormalities (Wang *et al*, 2016a).

Similar results have been reported by DTI studies in traumatic brain injury (TBI) patients. For instance, one study reported that the amount of damage in the SN white matter tracts i.e. tracts connecting the AI to the dACC and the pre-supplementary motor areas predicted abnormalities in DMN functioning in patients with cognitive impairments following TBI (Bonnelle *et al*, 2012). Another follow-up study showed that damage to the SN white matter tracts

was related to aberrant patterns of connectivity between the SN and the DMN in TBI patients (Jilka *et al*, 2014). Taken together, the evidence indicates that connectivity between key SN nodes could prove to be a useful biomarker in understanding how SN interacts with and modulates other brain networks.

To date, most functional imaging studies have used Fc based methods to investigate connectivity within the SN. However, Fc-based methods are only capable of examining the pattern of instantaneous correlations between time-courses obtained from two regions and are unable to investigate the directions of influence between brain regions or networks. Effective or causal connectivity methods on the other hand are beneficial as they can be used to understand the causal relationships that may exist across networks and in this case, how these relationships are affected in patients with schizophrenia. Three studies, mentioned previously, used an effective connectivity technique called Granger Causality Analysis (GCA) in order to demonstrate abnormal patterns of causal connectivity between the SN and CEN in patients with schizophrenia (Palaniyappan *et al*, 2013; Moran *et al*, 2013; Manoliu *et al*, 2014). GCA is a useful technique to measure causal relationships between brain regions at rest and a review paper on the functioning of the salience network emphasized the importance of this work and stated that these GCA results have provided the most convincing evidence of SN dysfunction in schizophrenia (Uddin, 2015). Although these studies have demonstrated abnormalities in causal connectivity between the SN and other key brain networks, to our knowledge, only one study so far has investigated abnormalities in within SN functioning in schizophrenia using effective connectivity methods. Schmidt and colleagues used Dynamic Causal

Modelling (DCM) and Bayesian model selection in task fMRI data and reported that unmedicated patients with first episode psychosis showed reduced reward-induced causal connectivity from the right insula to the ACC compared to healthy controls (Schmidt *et al*, 2016). Although it is interesting to investigate causal interactions within the SN during task performance; in patient cohorts particularly, it is quite useful to explore these interactions at rest. There are several advantages of resting-state fMRI – assessing brain function without the need for an explicit task removes the confounding influence of task performance and any systematic differences between groups in task accuracy, reaction time etc. Moreover, any variation in psychological strategies among individuals when performing cognitive tasks is also mitigated by using resting-state fMRI.

Therefore, in this analysis, we primarily aimed to extend upon previous work and used region of interest (ROI)-wise GCA in the resting-state to explore the effective connectivity between the key SN nodes – bilateral insula and the dACC in healthy controls and patients with schizophrenia. Briefly, Granger Causal (GC) connectivity is a time-lagged autoregressive model to quantify the effect one neural system exerts over another and the presence of GC connectivity from a region A to another region B implies that the neuronal activity in region A precedes and predicts the neuronal activity that occurs in region B. These two regions, A and B, may not show instantaneous functional coupling that is characteristic of a single large-scale system (Palaniyappan *et al*, 2013).

Although GCA is an increasing popular method to study effective connectivity, its validity using fMRI data is often contested. The main concern is related to the possible confounding effects of hemodynamic delay and the variability of the hemodynamic response function (HRF) which fMRI responses are convolved to, between different brain regions. This issue has been discussed in detail in a previously published paper from our group (Palaniyappan et al, 2013). Briefly, although results using modelled data showed that the identification of causal influences was only slightly above chance when hemodynamic delay varied randomly between subjects (Smith *et al*, 2011); a study using real data showed that GCA identified causal influences in group studies with good sensitivity and specificity, indicating that the observed causal effects are most likely to be neural (Schippers *et al*, 2011). Moreover, many studies have demonstrated that consistent effects can be obtained using GCA of BOLD data (e.g. Sridharan *et al*, 2008; Deshpande *et al*, 2011; Palaniyappan *et al*, 2013, Moran *et al*, 2013). This implies that the random variation between subjects in hemodynamic delay (modelled by Smith *et al*, 2011) are not large enough obliterate systematic effects. Though, the concern is that systematic group differences observed in GCA analyses of BOLD data could reflect systematic group differences in either in neural activity, in hemodynamic response or in both.

Recently, Wen and colleagues used simulation experiments to investigate whether changes in Granger Causality observed using fMRI are related to changes in neural Granger Causality (Wen *et al*, 2013). They found that Granger Causality from the HRF convolved time series is a monotonic function of Granger Causality from the underlying neural time series, an effect

which was preserved despite severe down-sampling and noise addition, suggesting that convolution with HRF has no adverse effects on Granger Causality estimation. While they also found that HRF latency variability degrades this detectability of monotonicity, they concluded that when HRF latency differences are unlikely to be systematic, group level Granger Causal influences detected by fMRI can reliably reflect neural changes (Wen *et al*, 2013). As explained in Palaniyappan *et al*. (2013), this conclusion is supported by the fact that the regions involved are served by different arteries and therefore group effects due to hemodynamic delay would only be expected if there were differences in arterial transmission times that were consistent across subjects.

Additional support for GCA comes from electrophysiological studies which have reported results consistent with GCA studies of BOLD data. For instance, a study using MEG demonstrated delay between the visual cortex and the insula (Brookes *et al*, 2012), comparable to results from a GCA study of BOLD data demonstrating a similar delay between the visual cortex and the insula (Palaniyappan *et al*, 2013). Similarly, Chand & Dhamala have reported causal interactions between SN and CEN nodes using GCA of electroencephalography (EEG) data, consistent with results reported by Sridharan *et al*. (2008) and other studies (Chand *&* Dhamala, 2016). Thus, in light of the consistency between electrophysiological evidence and BOLD findings using GCA, it is likely that in at least some circumstances, the CGA findings do reflect neural delay.

In a report on the use of GCA in neuroscience and neuroimaging, Seth *et al*. concluded GCA is a powerful and statistical tool but that applications of GCA to fMRI data should be treated with caution and experimental paradigms with comparisons between conditions and/or groups wherein HRFs can be assumed to be unchanged should be used (Seth *et al*, 2015). Thus, in summary, although GCA using fMRI data has been regarded as controversial; recent evidence suggests that differences in Granger Causal influences between groups of subjects or experimental conditions reflect real neural effects and are valid. If used appropriately, GCA can be a very valuable neuroimaging technique to understand the flow of information in key neural circuits.

In this analysis, we hypothesized that patients with schizophrenia will show aberrant patterns of causal interactions between key nodes of the SN compared to healthy controls. Given the vast amount of evidence emphasizing the importance of SN integrity in schizophrenia, understanding the causal mechanisms that govern within SN interactions could provide further insight into understanding the pathophysiology of schizophrenia. The second research question addressed in this work relates to whether any abnormalities in causal connectivity between key SN nodes are related to neurochemical abnormalities within the SN in schizophrenia patients. As discussed in detail in chapter 4, several studies have reported neurochemical abnormalities in the ACC in patients with schizophrenia (Tayoshi *et al*, 2009; Egerton *et al*, 2012; Bustillo *et al*, 2014; Plitman *et al*, 2016; for reviews see Poels *et al*, 2014; Wijtenburg *et al*, 2015). However, a direct investigation of if and how these abnormalities relate to SN functioning has yet to be

undertaken. Moreover, to our knowledge, no studies have investigated neurochemical levels in the insula using MR spectroscopy in schizophrenia. However, similar questions have been addressed in major depressive disorder (MDD). A study by Horn and colleagues reported that Glx (glutamate (Glu) + glutamine (Gln)) levels in the ACC and not the left AI predicted Fc between these two regions in the resting-state in depressed patients (Horn *et al*, 2010). This work provides support for the idea that neurochemical concentrations in the ACC and not the insula could be related to the functioning of the SN. Given the structural connections between the insula and the ACC, it is plausible that neurochemical concentrations in the ACC have a relationship to the functioning of the insula and the SN as whole; and if these results are replicated in patients with schizophrenia, they would be in line with the large body of evidence (including the work presented in chapter 4) showing abnormal neurochemical levels in the ACC in schizophrenia. However, it is also worth noting that a study in patients with chronic pain demonstrated that increased Glx levels in the AI in patients correlated with increased Fc between the AI and mPFC (As-Sanie *et al*, 2016). Therefore, the potential role for insula neurochemical concentrations in the functioning of the SN should not be ignored.

Nevertheless, in this cohort, as reported in chapter 4, we found that the shared variance between GSH and Glu in the ACC differentiated all patients with schizophrenia from healthy controls, and also differentiated patients with residual schizophrenia from other schizophrenia patients and healthy controls. However, a direct investigation of if and how these abnormalities relate to SN functioning has yet to be undertaken. Hence, in this analysis, we aimed to

investigate if aberrant causal interactions within the SN in schizophrenia are correlated with reduced GSH-Glu levels in the ACC. Understanding the link between the neurochemistry and functioning of the SN will allow us to further understand the mechanistic process by the SN modulates the functioning of other brain networks as well as the nature of SN dysfunction in schizophrenia.

5.3. Methods

5.3.1. Participants

Details relating to volunteer recruitment and clinical and cognitive assessments for this dataset have been reported in detail in chapter 2, section 2.1, and summarized in chapter 3, section 3.3.1.

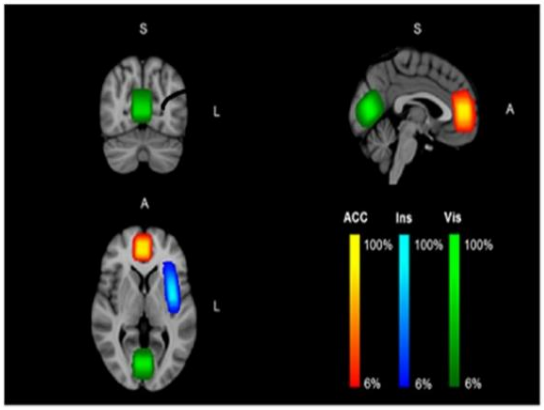
5.3.2. Image acquisition

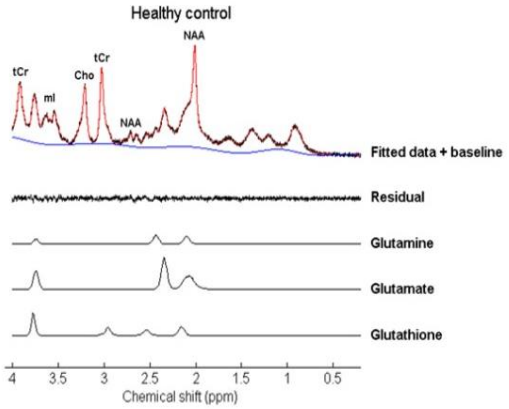
5.3.2.1. Resting-state fMRI

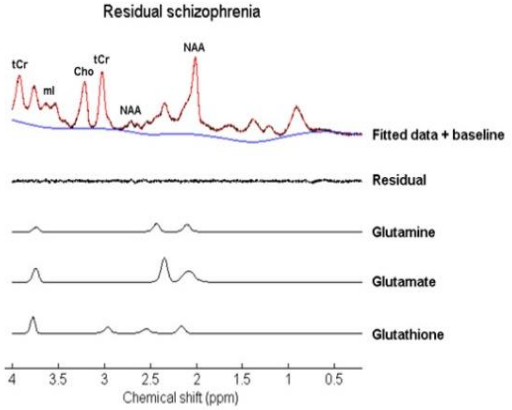
All scans were acquired at the Sir Peter Mansfield Imaging Centre, University of Nottingham. A 7 Tesla Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) was used. The resting-state scan lasted for approximately 5 minutes and the multi-slice gradient echo-planar images (EPI) were acquired using a 32-channel head coil with SENSE factor 2.5 and the following parameters: TE = 25 ms, TR = 2000 ms, flip angle = 75°, in plane resolution = 2x2mm, slice thickness = 2mm and a TR of 2000 ms. 150 dynamic time-points were acquired with each volume consisting of 36 contiguous axial slices in ascending order. During the scan, participants were asked to keep their eyes open and focus on a fixation cross on the screen. An anatomical T1 MPRAGE image was acquired for each subject using the following parameters: 1mm isotropic resolution, 256x256x180 matrix, flip angle 8°, TE/TR=3.4/7.3ms for image registration.

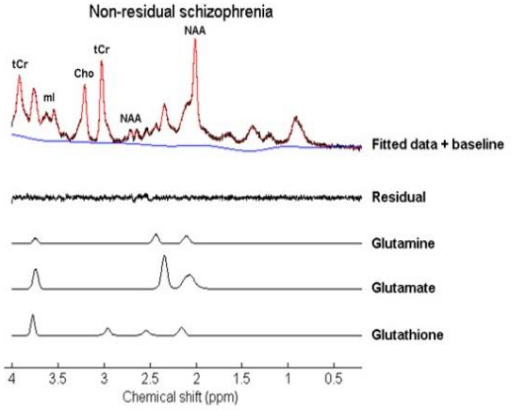
5.3.2.2. MR Spectroscopy

MRS scans were also acquired at the Sir Peter Mansfield Imaging Centre, University of Nottingham. Details regarding MRS data acquisition have been reported in detail in chapter 4. Briefly, MRS data were collected using single voxel 1H-MRS at 7T using a STEAM sequence and the following parameters (TE/TM/TR=17/17/2000ms, phase cycle steps = 8, samples = 4096, bandwidth = 4 kHz). 288 spectra were collected and the MOIST technique was used for water suppression. Three VOIs were used (ACC = (20x18x25mm3), left insula = (40x12x18mm3) and the visual cortex = (20x22x20mm3)). Sample spectrum and voxel placements have been presented in chapter 4 section 4.3.2 previously and have been reproduced here. An anatomical T1 MPRAGE image was acquired for each subject (described in section 5.3.2.1) and was used to aid placement of the VOIs and for co-registration.


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6%
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Healthy control
tCr
ml
tCr
Cho
NAA
NAA
Fitted data + baseline
Residual
Glutamine
Glutamate
Glutathione
4
3.5
3
2.5
2
1.5
1
0.5
Chemical shift (ppm)


Residual schizophrenia
tCr
ml
Cho
tCr
NAA
NAA
Fitted data + baseline
Residual
Glutamine
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4
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Chemical shift (ppm)


Non-residual schizophrenia
tCr
ml
Cho
tCr
NAA
NAA
Fitted data + baseline
Residual
Glutamine
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Chemical shift (ppm)

5.3.3 Data analysis

5.3.3.1. Resting-state fMRI pre-processing

The resting-state scans were pre-processed using Data Processing Assistant for Resting-State fMRI (Chao-Gan *&* Yu-Feng, 2010) and the Data Processing & Analysis for Brain Imaging (DPABI) toolbox (Yan *et al*, 2016) using a standard pre-processing pipeline. The first 5 time points of the data were discarded due to the instability of the initial signal, leaving 145 data points for further analysis. Slice time-correction was undertaken and data were then spatially realigned to the first image of the dataset. Movement parameters were calculated for each subject and a stringent approach was adopted in order to correct for head motion. Subjects with a high degree of head motion (>3mm and 3 degree) were excluded and the Friston 24 approach was used in order to correct for head motion effects. This model uses 24 parameters to perform the correction - the 6 head motion parameters, the 6 head motion parameters one time point before, and the corresponding 12 squared values (Friston *et al,* 1996). This approach was utilized as it has been shown to be superior to other similar approaches in terms of reducing the link between head motion and the BOLD signal in individual subjects (Yan *et al*, 2013). The linear trend was removed and nuisance covariates white matter and cerebrospinal fluid signal were regressed out before further processing using the CompCor approach (Behzadi *et al*, 2007).

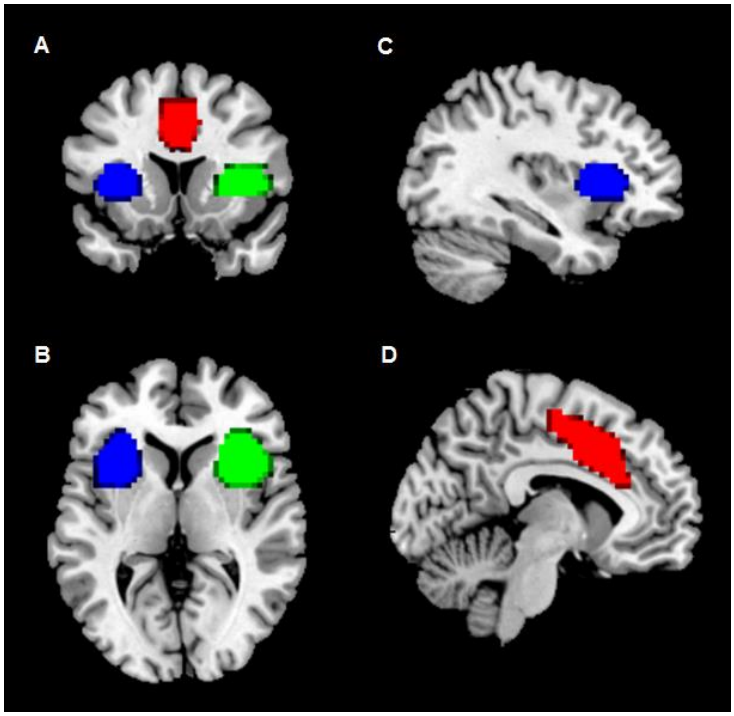
Spatial normalization using each participant's anatomical image was carried out using the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) technique (voxel size 3×3×3mm) and the functional

scans were smoothed using a Gaussian kernel of 8mm full-width at half-maximum. Next, the functional data were filtered using a band-pass filter (0.01–0.08 Hz) in order to eliminate low-frequency fluctuations and high-frequency noise. Finally, scrubbing was performed in order to correct the data for micromovements and further reduce the effects of spurious correlations using frame-wise displacement (0.5mm) and a nearest neighbour interpolation approach (Power *et al*, 2012).

5.3.3.2. ROI selection

Since our primary aim was to investigate causal connectivity within key SN nodes, the ROIs for the GCA analysis were defined using BOLD activation data in the same cohort during the relevance modulation task (mentioned in chapter 3). The results from the one-sample t test for the relevant vs irrelevant condition (familywise error [FWE] corrected $p < 0.05$, described in chapter 3, section 3.4.3.) were used and the bilateral AI and dACC ROIs were extracted from these activation maps and converted into binary masks. These have been presented in Figure 11. Thus, the ROIs chosen to explore the causal connectivity within the SN were defined by using activation data which corresponded to areas of the SN involved in differentiating relevant from irrelevant stimuli in this cohort.

Figure 11: Binary region of interest (ROI) masks used for the ROI-wise Granger Causality Analysis overlaid on a standard skull stripped MNI brain.*



* A = left anterior insula (blue), right anterior insula (green) and dorsal anterior cingulate cortex (dACC) (red) masks in coronal view, B = left and right anterior insula masks in axial view, C = left anterior insula mask in sagittal view, D = dACC mask in sagittal view. Masks were defined using BOLD activation data in the same cohort during the relevance modulation task (mentioned in chapter 3). The results from the one-sample t test for the relevant vs irrelevant condition (familywise error [FWE] corrected $p < 0.05$, described in chapter 3, section 3.4.3.) were used and the bilateral AI and dACC ROIs were extracted from these activation maps and converted into binary masks.

5.3.3.3. Granger Causality Analysis: single subject level

Granger Causality proposes that a time series (X) exerts a causal influence on another time series (Y) if past values of X can help predict the future values of Y, better than past values of Y alone. Granger Causality can be performed at the whole-brain level i.e. estimates the causal effect of the seed region of interest (X) on every other voxel in the brain (Y) (X to Y effect), as well as the causal effect of every voxel in the brain on the seed region of interest (Y to X effect). It can also be performed between pre-specified regions of interest. In this case, we used ROI-wise GCA to investigate the causal influence between three ROIs – left AI, right AI and dACC. Similar to the voxel-wise GCA, a positive coefficient from X to Y indicates that activity in region X exerts a causal influence on the activity region Y in the same direction (i.e. positive influence). Similarly, a negative coefficient from Y to X suggests that the activity of region Y exerts an opposing directional influence on the activity of region X (i.e. negative influence). Using this approach, we are able to build a Granger-causal model based on the temporal elements of regional BOLD activity between three X to Y pairs (left AI and right AI, left AI and dACC, right AI and dACC) in this instance. GCA analysis was performed using the REST software (Song *et al*, 2011) and bivariate, co-efficient based, ROI-wise analyses were undertaken to investigate the causal influences between the nodes of the SN.

5.3.3.4. Granger Causality Analysis: group level

The signed path co-efficients for X-to-Y and Y-to-X directions for each ROI pair were extracted from the output files of the first level REST analysis and

examined further at group level using SPSS 22 (IBM). Independent sample t-tests were used to determine if there were any significant differences in Granger Causal influence within the SN between schizophrenia patients and controls. All analyses were bootstrapped using 10,000 samples and Bias-Corrected accelerated (BCa) *p* values were used to report results.

5.3.3.5. MR Spectroscopy data processing

The processing pipeline for the MRS data has been described in detail in chapter 4, section 4.3.3. In summary, segmentation of the structural MRI image was undertaken in SPM and tissue volume fractions for grey matter, white matter and CSF within each voxel were calculated. MRS data were reformatted, phase-corrected, realigned and then joined together (Hall *et al*, 2014). LCmodel was used in order to estimate metabolite concentrations (Provencher, 1993). A custom basis set which included 20 metabolites was used for the estimation procedure and lipid resonances and macromolecules were also included in the fit. Water concentrations from the unsuppressed water spectra were used as the concentration reference and the reliability of the model fit was assessed using Cramér-Rao Lower Bound (CRLB) values. Data above a CRLB threshold of SD=20% were excluded from the analysis (Provencher, 2015). Metabolite concentrations were then corrected for partial volume effects and relaxation attenuation in the voxel using a method described by Gasparovic and colleagues (Gasparovic *et al*, 2006).

5.3.3.6. MR Spectroscopy statistical analyses

Statistical analyses performed on the MRS data have been explained in detail in chapter 4, section 4.4. Briefly, after adjusting for potential confounds; glutathione, glutamate and glutamine concentrations from the three voxels were entered into a PCA in order to extract non-collinear predictors. This approach was adopted due to significant correlations between glutathione and glutamate within each voxel, and significant correlations between glutamine concentrations across voxels. The PCA resulted in three principal components – an ACC glutathione-glutamate component, a three voxel glutamine component and a visual-insula glutathione-glutamate component. The component loadings from the PCA have been presented in chapter 4, table 9. Next, the component scores from the PCA were entered into hierarchical binary logistic regression analyses in order to examine the relationship between metabolite concentrations and diagnosis. This analysis demonstrated that the ACC glutathione-glutamate component was a significant predictor in differentiating all patients from schizophrenia from healthy controls, with lower levels being associated with a raised probability of schizophrenia. Additionally, this component was also a significant predictor in differentiating patients with residual schizophrenia from healthy controls and other schizophrenia patients, with lower levels associated with an elevated probability of residual schizophrenia.

5.3.3.7. Correlation between within-SN GC influence and MRS measures

As mentioned in the introduction, we then sought to examine if neurochemical changes within the SN are related to within-SN effective connectivity

measures. Since the ACC glutathione-glutamate component was a significant predictor in differentiating between all schizophrenia patients and controls, a bivariate Pearson correlation (bootstrapped, 10,000 samples) was undertaken to investigate if this neurochemical abnormality was related to any aberrant causal interactions within the SN in patients with schizophrenia (identified using the independent samples t-test of GCA measures, described in section 5.3.3.5).

5.3.3.8. Correlation between within-SN GC influence and clinical confounders

Using the same approach described in section 5.3.3.8 above, we also examined the relationship between any abnormal causal influences within the SN in schizophrenia patients and medication (antipsychotics, antidepressants and mood stabilizers DDD) and duration of illness.

5.4. Results

5.4.1. Participant inclusions and exclusions

From the 46 healthy participants and 41 patients with schizophrenia recruited for this study, 44 healthy controls and 25 patients with schizophrenia were included in the analysis. 2 healthy controls and 14 patients with schizophrenia did not undergo fMRI data acquisition and hence were not included in the analysis. 2 patients were excluded due to excessive movement. MRS data were analysed with the entire available sample (as described in chapter 4, section 4.4.1.) and data for the participants who had both fMRI and MRS data (22 patients, 41 controls) were used for the correlation analysis. Clinical and demographic features of the sample are reported in Table 10.

Table 10: Clinical and demographic features of the sample

Features	fMRI Schizophrenia Patients (N = 25)	fMRI Healthy Controls (N = 44)	MRS Schizophrenia Patients (N = 28)	MRS Healthy Controls (N = 45)
	Mean/N (SD)			
Gender (Male/Female)	18/7	29/15	20/8	29/16
Age	27.08 (6.82)	27.43 (6.97)	27.18 (6.54)	27.89 (7.54)
Parental NS-SEC	2.40 (1.80)	1.90 (1.37)	2.39 (1.72)	1.97 (1.43)
Mean Illness Duration	53.08 (47.27)	-	54.42 (48.43)	-
DDD Antipsychotics	1.21 (0.72)	-	1.21 (0.69)	-
DDD Mood Stabilizers	0.06 (0.24)	-	0.05 (0.22)	-
DDD Antidepressants	0.48 (0.95)	-	0.43 (0.91)	-
SOFAS Score*+	58.44 (16.31)	88.70 (5.88)	57.35 (16.25)	88.73 (5.82)
Quick IQ*+	98.72 (9.39)	104.25 (9.54)	97.82 (9.66)	104 (9.69)
Anxiety/Depression*+	1.52 (1.19)	0.77 (0.85)	1.57 (1.23)	0.78 (0.85)
Reality Distortion*+	2.04 (2.44)	0.14 (0.55)	2.11 (2.40)	0.13 (0.54)
Psychomotor Poverty*+	2.20 (1.82)	0.25 (0.48)	2.29 (1.88)	0.24 (0.48)
Disorganization	0.40 (0.57)	0.20 (0.55)	0.46 (0.63)	0.20 (0.54)
Psychomotor Excitation	0.60 (1.08)	0.43 (0.81)	0.57 (1.03)	0.47 (0.84)

NS-SEC: National Statistics Socio-Economic Classification; Symptom scores computed from SSPI: Signs and Symptoms of Psychotic Illness scale; DDD: Defined Daily Dose; SOFAS: Social and Occupational Functioning Assessment Scale. Mean Illness Duration reported in months

* = significant difference between healthy controls and schizophrenia patients in the fMRI dataset ($p < 0.05$)

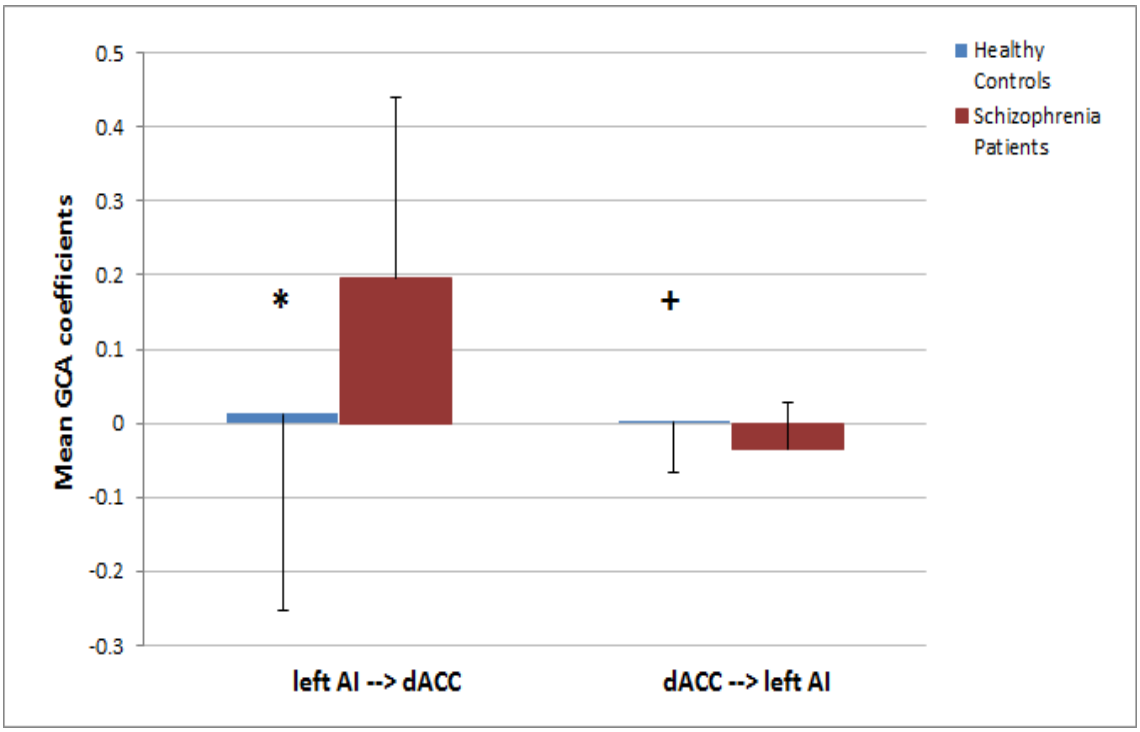
+ = significant difference between healthy controls and schizophrenia patients in the MRS dataset ($p < 0.05$)

5.4.2. Granger Causality Analysis

Results from the independent samples t-tests revealed significant differences in GC influence from the left AI to the dACC (bootstrapped p = 0.005) and also from the dACC to the left AI (bootstrapped p = 0.030) between healthy controls and patients with schizophrenia. There was also a trend toward a significant difference in GC influence from the right AI to the dACC (bootstrapped p = 0.084).

Additional analyses using one sample t-tests revealed that in healthy controls, there were no significant causal influences between the left AI and the dACC at rest. However, in patients with schizophrenia, the left AI exerted a significant positive causal influence on the dACC (bootstrapped p=0.002) and the dACC in turn exerted a significant negative causal influence on the left insula (bootstrapped p=0.015). Mean GCA co-efficients in both directions (left AI → dACC and dACC → left AI) in the two groups are shown in Figure 12. Since the GC influence from the right AI to the dACC showed a trend toward significance, we also performed follow-up one sample t-tests to further explore this effect. In healthy controls, there were no significant causal influences between the right AI and the dACC. On the other hand, in patients with schizophrenia, similar to the left AI, the right AI also exerted a significant positive causal influence on the dACC (bootstrapped p = 0.003) and the dACC showed a trend toward exerting a negative causal influence on the right AI (bootstrapped p = 0.065). Please see Figure 13 for a graphical representation of these results.

Figure 12: Mean GCA co-efficients from the left AI → dACC and dACC → left AI in the two groups – healthy controls and patients with schizophrenia

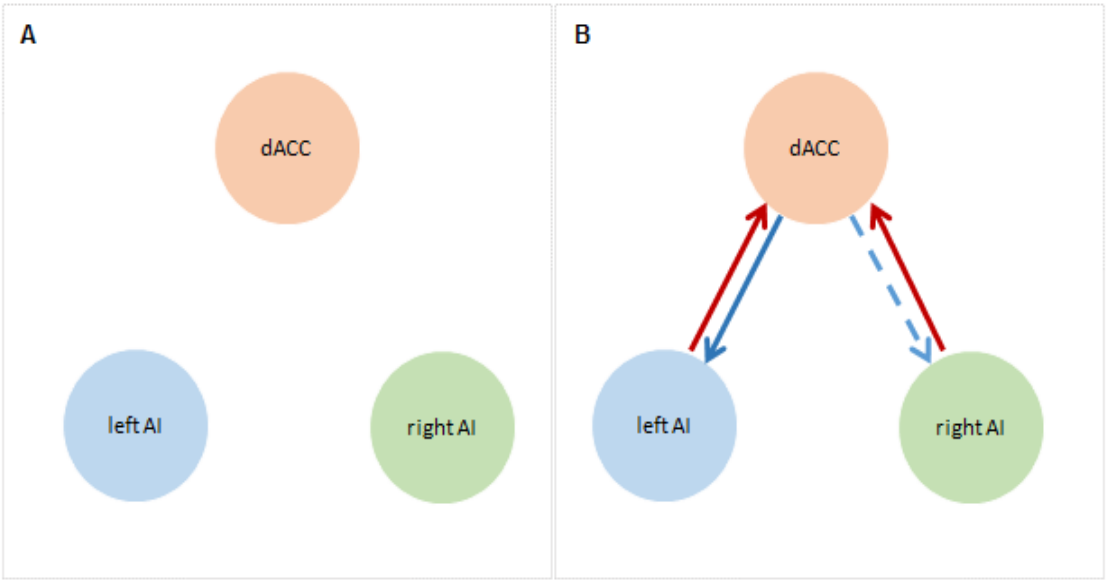


Mean GCA co-efficients representing magnitude and direction of effective connectivity between the left anterior insula (AI) and the dorsal anterior cingulate cortex (dACC). In healthy controls, there are no significant causal interactions between the left AI and the dACC. Patients with schizophrenia exhibit a significant positive causal influence from the left AI to the dACC and a significant negative causal influence from the dACC to the left AI.

* = significant difference between healthy controls and schizophrenia patients (bootstrapped $p = 0.005$)

+ = significant difference between healthy controls and schizophrenia patients (bootstrapped $p = 0.030$. Error bars represent standard deviations.

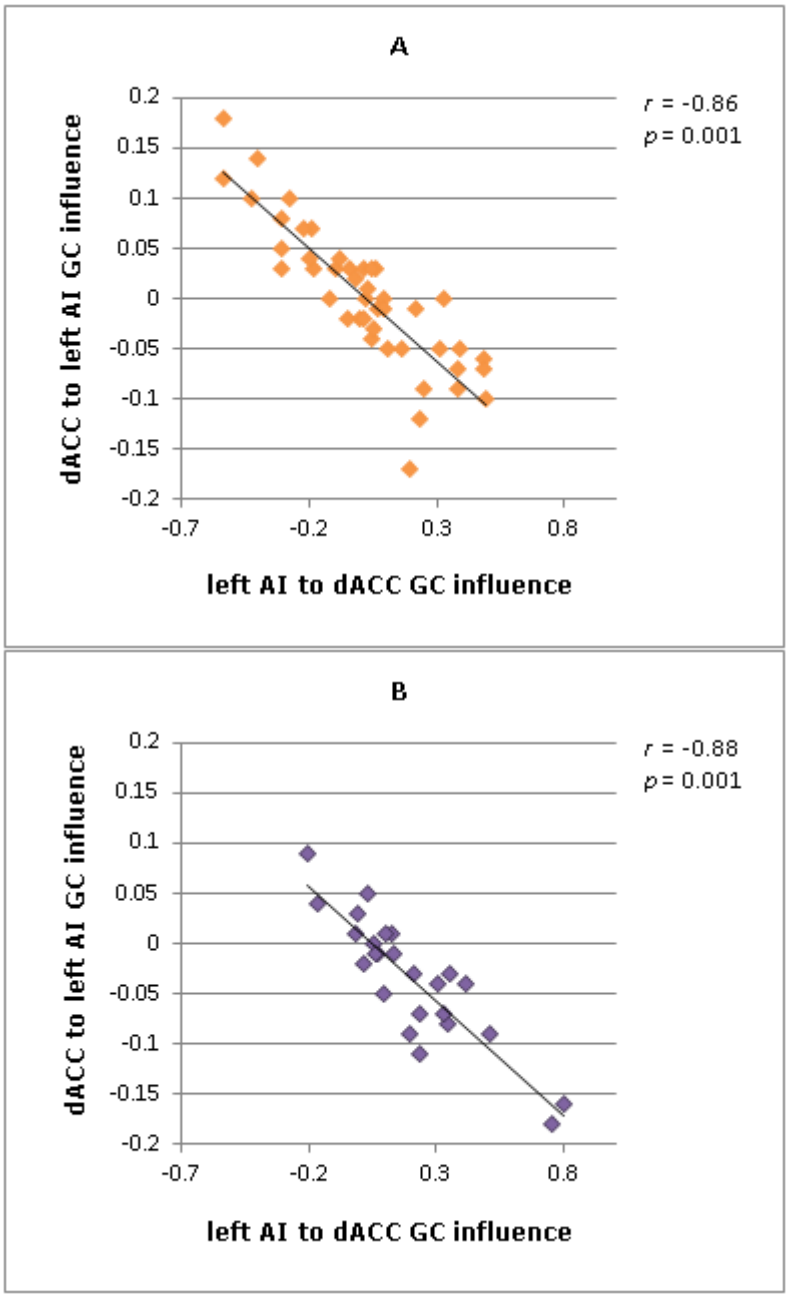
Figure 13: A graphical representation of effective connectivity between the three key nodes of the SN = left AI, right AI and dACC in the two groups (A = healthy controls, B = patients with schizophrenia).*



* Coloured lines represent direction of causal influence: Red lines indicate statistically significant positive causal connectivity and blue lines indicate statistically significant negative causal connectivity. Solid lines indicate significant causal connections in a group (one sample t-test results, bootstrapped $p < 0.05$) and dashed lines indicate a trend toward a significant effect.

Since the causal influence between the left AI and the dACC was statistically significant in both directions in patients with schizophrenia, we sought to examine if there is a reciprocal effective connectivity 'loop' between these two regions. To this effect, we examined the correlation between left AI → dorsal ACC GC influence and dACC → left AI GC influence using bivariate Pearson correlation. In schizophrenia patients, we found a significant negative correlation between the GC influence from the left AI → dACC and the GC influence from the dACC → left AI in schizophrenia patients (r = -0.88, p < 0.001). These results indicate that in patients with schizophrenia, greater positive causal influence from the left AI → dACC was associated with a greater negative causal influence from the dACC → left AI. Despite no significant group-level causal influence between these two regions in healthy controls, there was nonetheless a significant negative correlation between GC influence from the left AI → dACC and the dACC → left AI in healthy controls (r = -0.86, p < 0.001), similar to that observed in schizophrenia. These results are presented in Figure 14.

Figure 14: Correlation between GC influence from the left AI → dACC and from the dACC → left AI in the two groups (A = healthy controls, B = patients with schizophrenia)

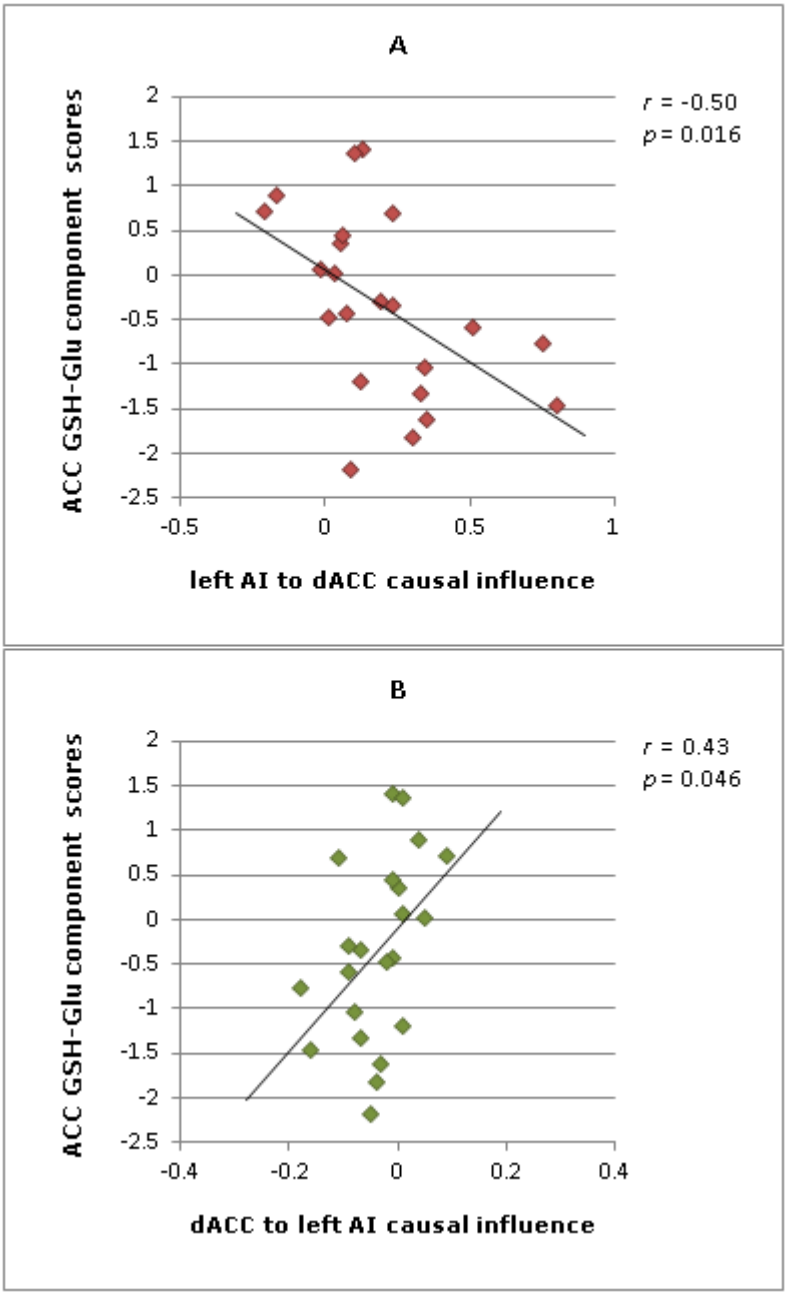


Greater positive causal influence from the left AI → dACC is associated with a greater negative causal influence from the dACC → left AI in both groups, indicating the presence of a reciprocal effective connectivity 'loop' between these two regions.

5.4.3 Correlation between GC and MRS measures

As explained in section 5.3.3.7, bivariate Pearson correlation was undertaken to investigate if abnormalities in causal connectivity between the left AI and dACC in patients with schizophrenia were correlated with the ACC glutathione-glutamate component. We found a significant negative correlation between left AI → dACC causal influence and the ACC glutathione-glutamate component scores (r = -0.508, p = 0.016) i.e. patients with greater positive causal influence from the left AI to the dACC had lower ACC glutathione-glutamate levels. We also found a significant positive correlation between dACC → left AI causal influence and the ACC glutathione-glutamate component (r = 0.430, p = 0.046) i.e. patients with lower positive causal influence (more negative causal influence) from the dACC to the left AI had lower ACC glutathione-glutamate levels. These results have been presented in Figure 15. No significant correlations were observed in healthy controls.

Figure 15: Correlation between GC and MRS measures in patients with schizophrenia.



A = correlation between GC influence from left AI → dACC and the ACC glutathione-glutamate (GSH-Glu) component scores. B = correlation between GC influence from the dACC → left AI and the ACC GSH-Glu component scores. Significant correlations between GC measures and the ACC GSH-Glu component scores demonstrate that patients with more aberrant patterns of causal influence between the left AI and dACC show greater reductions in glutathione and glutamate levels in the ACC.

5.4.4. Correlation between GC measures and clinical confounders

We found no significant correlations between causal interactions from the left AI → dACC or from the dACC → left AI and the DDD for antipsychotics, antidepressants or mood stabilizers. We also found no significant correlations between these GC measures and duration of illness in patients.

5.5. Discussion

In this study, using resting-state GCA, we primarily aimed to investigate if there are abnormalities in causal interactions within the SN in schizophrenia patients. Our results demonstrate that there are significant differences in causal connectivity between the left AI and the dACC between healthy controls and patients with schizophrenia. Healthy controls showed no significant causal interactions between these two SN nodes. As this was a resting-state paradigm in which participants had to focus on a static fixation cross on the screen and not attending to any salient stimuli, no significant causal interactions between SN nodes in healthy controls was an expected result. However, under the same conditions, in patients with schizophrenia, the left AI exerted a significant positive causal influence on the dACC and the dACC exerted a significant negative causal influence on the left AI i.e. the left AI ‘switched on’ the dACC and the dACC ‘switched off’ the left AI. Similar effective connectivity patterns were observed between the right AI and the dACC, although there were no statistically significant differences between the two groups.

These findings are intriguing and to our knowledge, this is the first study to demonstrate abnormalities in effective connectivity within the SN in schizophrenia.. As these results were observed in the resting-state, despite the absence of externally salient task based stimuli, it is plausible that patients with schizophrenia engage the SN at rest in order to process internally salient but inconsistent thoughts and feelings. Given the large body of evidence demonstrating the important role played by the ACC in error detection, conflict

monitoring and cognitive control (Kerns *et al*, 2004; Spunt *et al*, 2012; Brown, 2013), the 'switching on' of the ACC at rest could be indicative of an increased effort to suppress conflicting and disorganized internal thoughts. In line with this, 'switching off' of the insula could possibly be indicative of decreased attention to externally relevant stimuli, in order to focus on suppressing conflicting internal thoughts. Previous work using Positron Emission Tomography (PET) has demonstrated significant correlations between disorganization syndrome scores in schizophrenia patients and reduced regional cerebral blood flow (rCBF) in the insular cortex and increased rCBF in the ACC (Liddle *et al*, 1992). Another study also demonstrated similar findings with positive correlations between ACC rCBF and disorganization in schizophrenia (Lahti *et al*, 2006). Additionally, a recent structural imaging study showed that reduced grey matter volume in the insula and increased grey matter volume in the dACC predicted the severity of negative formal thought disorder in patients with schizophrenia (Palaniyappan *et al*, 2015b). As most structural imaging studies report decreases in grey matter volume in schizophrenia, this counter-intuitive increase in grey matter volume in the dACC was interpreted by the authors as an 'inefficient and inadequate compensatory response to formal thought disorder'. Our results are in line with these findings and are suggestive of an attempt at a similar compensatory response in patients.

Furthermore, the finding that aberrant effective connectivity between the left AI and the dACC is related to the ACC glutathione-glutamate component lends further support to this theory. We found a significant negative correlation between the causal influence from the left AI to the dACC and the ACC

glutathione-glutamate component indicating that patients with more abnormal causal influence i.e. increased positive causal influence from the left AI to the dACC had reduced glutathione and glutamate in the ACC. Similarly, we also found a significant positive correlation between the causal influence from the dACC to the left AI and the ACC glutathione-glutamate component demonstrating that patients with increased aberrant effective connectivity i.e. increased negative causal influence from the dACC to the left AI also had reduced glutathione and glutamate in the ACC. As reported in chapter 4, the ACC glutathione-glutamate component was a significant predictor of residual schizophrenia, characterized by long-term negative symptoms and impairments. Although there were no statistically significant differences in disorganization syndrome scores between the residual and non-residual schizophrenia groups (chapter 4, section 4.4.1, table 4), mean disorganization syndrome scores were higher in residual schizophrenia patients. Therefore, the observation that patients with more abnormal patterns of causal influence within the SN have reduced glutathione and glutamate in the ACC supports the notion that schizophrenia patients engage the SN at rest in order to deal with disorganized and conflicting thoughts. The 'switching on' of the ACC and 'switching off' of the insula in these patients could lead to reduced attention toward and interest in the external world, leading to anhedonia, poverty of speech and poor socio-occupational functioning.

We also found a significant negative correlation between effective connectivity from left AI → dACC and dACC → left AI in both patients and healthy controls. Thus, the change from negative and increase toward positive causal influence from left AI → dACC was associated with the change from positive and

increase toward negative causal influence from dACC → left AI. The fact that this reciprocal relationship was also observed in healthy controls, despite no significant causal connections between these nodes at group level, lends support that to the idea these key SN nodes rely on each other to modulate effective SN functioning. Therefore, even in the healthy brain, the left AI and the dACC maintain a stable effective connectivity loop at rest. However, in patients, particularly in those with more residual symptoms, this loop gets over-engaged, potentially in an attempt to deal with disorganized and conflicting internal thoughts.

In this study, using high resolution 7T fMRI and GCA, we demonstrate that patients with schizophrenia abnormally engage the SN at rest. Additionally, these results also show that abnormal causal interactions within SN nodes are related to glutathione and glutamate levels in the ACC. Thus, similar to the findings by Horn *et al*. in depression (Horn *et al*, 2010), our results demonstrate that neurochemical levels in the ACC are related to the functioning of the SN in schizophrenia patients. These results are novel and improve our understanding of SN dysfunction in schizophrenia, and how it related to neurochemical abnormalities within the SN and symptomatology.

However, a few limitations of this study must be taken into account while interpreting these findings. Firstly, as mentioned previously in chapters 3 and 4, the sample size of the patient group was modest. Studies with larger sample sizes would be required in order to corroborate these findings, particularly in relation to investigating the link between residual symptoms, disorganization and SN functioning. The validity of using GCA to examine

effective connectivity in the brain has been discussed in section 5.3.3.3. However, the technique still remains controversial and it would be of interest to aim to replicate these findings using other effective connectivity approaches such as DCM. Moreover, it is important to note that the ACC ROI used in the GCA analysis was dorsally located as compared to the ACC MRS voxel which was positioned in the medial prefrontal cortex. Therefore, although we found significant correlations between ACC neurochemical levels and causal interactions within the SN in this study, it would be of interest to investigate how neurochemical levels in the dACC relate to SN functioning. Lastly, although we found no significant correlations between current medication intake and GC measures, the long-term effects of psychotropic medication on the functioning of the SN cannot be ruled out.

In summary, this analysis has revealed interesting findings and follow-up studies must be undertaken in order to explicitly test whether these abnormalities in causal interactions within the SN are specific to patients with increased disorganization or formal thought disorder. It is plausible that patients with different symptom profiles might exhibit variations in causal influences within the SN, as compared to the results observed in this cohort. Additionally, it would also be useful to investigate if there are any differences in resting-state effective connectivity within the SN between patients in the acute psychotic phase of the illness and those in the chronic phase. Longitudinal studies are required in order to unravel the complex relationships between neurochemical abnormalities, SN functioning and symptomatology in schizophrenia.

Chapter 6

Conclusions and future directions

This thesis investigated of the neurobiological basis of Salience Network (SN) dysfunction in schizophrenia. The four main aims of this work were: 1) to examine how salience affects the SN and the brain in schizophrenia, 2) to investigate whether neurochemical abnormalities in schizophrenia differ according to the clinical status of patients and 3) to study the within SN dysconnectivity in schizophrenia and 4) to examine the relationship between within SN dysconnectivity and neurochemical abnormalities. To this effect, we employed multi-modal neuroimaging techniques (task and resting-state fMRI, MEG and MRS) in a cohort of healthy volunteers and stable medicated patients with schizophrenia. The main findings are summarized below, and recommendations for future work presented.

Firstly, using task-based fMRI, we found that relevant stimuli strongly activated the SN and other task-positive brain areas including CEN regions, and also produced significant suppression of DMN areas compared to irrelevant stimuli, in the entire sample. After controlling for differences in task performance, we found significantly increased activation in CEN areas and increased suppression in DMN areas in patients with schizophrenia during the processing of relevant stimuli compared to irrelevant stimuli. We concluded that in order to achieve the same level of performance as healthy controls, patients with schizophrenia tended to over-engage the CEN and also over-

suppress the DMN. Thus, in order to effectively attend to and process salient stimuli, patients need to recruit additional neuronal resources, however, are they able to do this efficiently?

The MEG analysis of this task revealed that patients with schizophrenia differ from healthy controls in their modulation of SN beta response to relevant stimuli compared to irrelevant stimuli, with a lower response to relevant stimuli in patients. We noted that these results i.e. reduced beta response in the SN along with increased BOLD in task positive areas and increased DMN suppression are broadly consistent with previous work relating BOLD activations to neural oscillations (Mantini *et al*, 2007; Ossandon *et al*, 2011). This work does support the inefficient cerebral recruitment hypothesis; however, as noted in the discussion section 3.5, additional analyses need to be performed with this data before any final conclusions are drawn. An initial inspection of the data has revealed a lot of variability both within and between subjects and trials. Therefore, in order to fully understand how this variability has impacted our results and given the evidence that beta occurs in transient bursts (Sherman *et al*, 2016); we need to re-analyse the data using single trial information. Another factor that requires consideration during the re-analysis of this data is the effect of medication. All patients in this analysis were medicated, and hence we could not inspect the effect of medication on our results by performing the analysis in a subset of patients, as done by Liddle *et al.* (2016). This work has the potential to provide more informative results, and the re-analysis of this data is ongoing.

In chapter 4, we reported that the shared variance between glutathione and glutamate in the ACC differentiated patients with residual schizophrenia from both healthy controls and other schizophrenia patients. This is an extremely important finding as it sheds some light on the cause of inconsistent findings in the MRS literature, given the heterogeneity in the patient groups examined by many studies. The fact that clinical features (and not just duration of illness) are directly related to the nature of neurochemical abnormalities in schizophrenia is a noteworthy result and will allow for more efficient study designs in the future.

The other interesting finding from this work is the correlation between glutathione and glutamate concentrations in the entire sample. This result is in line with the suggestion that there is a mechanistic link between these two systems; however further exploration of this theory using more direct measures of NMDAR hypofunction e.g. by using PET ligands as suggested by Hardingham *&* Do (2016) are required. In relation to the SN, future studies should aim to investigate neurochemical concentrations in all three major SN nodes. Moreover, appropriate voxel placement is certainly an important issue to consider, as mentioned previously in chapter 4. A follow-up MRS study using four voxels – right insula, left insula, dACC and mPFC would be beneficial in terms of exploring the precise nature of neurochemical dysfunction in the SN in schizophrenia; however the practicalities of implementing this in a multi-modal imaging protocol also need to be considered.

Finally, in chapter 5, using GCA, we found that effective connectivity within the SN (between the left AI and the dACC) is disrupted at rest in schizophrenia patients. These results indicate a loss of integrity and coherent functioning within the SN in schizophrenia, and could have implications for its connections with other brain regions and networks. This hypothesis could be tested in future studies and a re-analysis of this dataset. As mentioned previously in chapter 5, due to the debate regarding the validity of GCA, these results need to be verified using other effective connectivity approaches such as Dynamic Causal Modelling (DCM) which are more suitable because they account for variations in the observed signal which might be due to haemodynamic delay (Friston, 2011). Recently, a new spectral DCM technique based on a deterministic model has been introduced for use with resting-state fMRI data. (Friston *et al*, 2014). This method has been used by a few recent studies to investigate effective connectivity at rest (Crone *et al*, 2015; Sharaev *et al*, 2016) and could be applied to our dataset. Additionally, using DCM, causal interactions between these SN nodes can also be analysed during the performance of the RM task, in order to directly examine the effect of proximal salience on the causal connectivity within the SN. In this chapter, we also found that abnormalities in the causal connectivity between the left AI and dACC in schizophrenia were related to the glutathione-glutamate component scores in the ACC. To our knowledge, this is the first attempt at investigating a link between neurochemical dysfunction and effective connectivity disturbances in the SN in schizophrenia and follow-up studies using multiple SN voxels and other causal connectivity methods can shed further light on how these two abnormalities are linked.

Overall, this thesis has contributed to our understanding of salience abnormalities in schizophrenia and provided a solid foundation for future studies in this area of research. A multi-modal imaging study design allowed us to investigate the neurobiology of schizophrenia using a wide range of measures, and also enabled us to investigate the link between various aspects of SN dysfunction. As mentioned by Kempton & McGuire, such multi-modal studies can be extremely useful as they can provide important complementary information which can be used to stratify patients prior to clinical trials (Kempton *&* McGuire, 2015). Heterogeneity in the symptoms and dysfunction observed in patients with schizophrenia has been a major issue in terms of translating research findings to clinical practice, and the development of new, effective medication. Therefore, if multi-modal imaging can contribute toward a better understanding of how this heterogeneity relates to differences in brain pathophysiology, this would be an important step forward towards translation.

The other important factor is medication use. Although most studies account for the effect of current medication use on imaging findings, we cannot exclude the effect of long-term medication use on brain physiology. There is some evidence to indicate that continued use of antipsychotics can result in progressive structural brain changes (Fusar-Poli *et al*, 2013), however the effect of long-term antipsychotics on neurochemical levels and brain connectivity is yet to be fully investigated. Disentangling the imaging correlates of medication use from disease-related changes is a complex issue and needs further consideration. In order to explore this issue effectively, it is important to obtain an accurate measure of the lifetime history of psychosis

and medication intake. We were only able to obtain this information for a subset of the patients in the MISP study, and further analyses of any data from this study could benefit from the inclusion of this information. Relatedly, it is also complicated to differentiate between compensatory neural responses and responses related to disease pathology, particularly in cases where there is partial compensation. In chapter 3 and 5, we have suggested that some of our findings are indicative of a compensatory response in patients; however, it is not straightforward to directly measure neural compensation. Another aspect of this work which could be improved in follow-up studies relates to the definition of residual schizophrenia. We have presented the list of criteria used to classify patients into the residual schizophrenia group; however, this could be developed further in future work.

Although this thesis focussed on SN dysfunction, overall, neuroimaging studies in schizophrenia offer a lot of potential in terms of providing findings with translational value and there are several research questions which can be addressed by future studies. One important line of work relates to the assessment of subjects at ultra-high risk (UHR) for psychosis. Can neuroimaging allow us to predict which UHR subjects will transition to psychosis or not? Evidence indicates that UHR subjects who go on to develop schizophrenia show differences in structural brain changes compared to those who not. For instance, one study demonstrated that UHR subjects who later developed schizophrenia demonstrated a greater grey matter reduction in the insula compared to UHR subjects who did not develop schizophrenia (Takahashi *et al*, 2009). Other studies have also reported progressive structural changes in UHR subjects who transition to schizophrenia (e.g. Job

et al, 2005; Wood *et al*, 2008). There is some evidence that early intervention in schizophrenia could lead to reduced conversion rates (McGorry *et al*, 2002; Schmidt *et al*, 2015). If we can accurately predict the onset of symptoms in the UHR group, effective pre-emptive treatment can be administered at an early stage to a subgroup that need it, before they develop full blown psychosis.

Another related research question with great translational value involves using neuroimaging to predict drug selection and response to treatment (Wooley & McGuire, 2005). Being able to accurately identify which medication is best suited to a patient will allow for clinicians to prescribe the right drug at first presentation of symptoms. Similarly, if neuroimaging studies can be used to predict response to treatment, this will help clinicians decide which patients will respond to treatment with antipsychotics. For instance, recently, it has been demonstrated that elevated ACC glutamate levels are observed in patients with treatment-resistant schizophrenia compared to those who responded to treatment (Mouchlianitis *et al*, 2016).

Recent advances in our understanding of the neurochemical pathways involved in schizophrenia have led to the development of new drugs. D-cycloserine, sacrosine and glycine are some of the glutamatergic drugs that have been developed over the decade, but evidence for the efficacy of these drugs is ambiguous. Although a few trials have shown that these drugs are moderately beneficial in improving specific cognitive domains or negative symptoms of schizophrenia (Heresco-Levy *et al*, 2004; Lane *et al*, 2010; Cain *et al*, 2014), these findings are yet to be widely replicated and large scale clinical trials are necessary in order to prove their efficacy. LY2140023 – a

mGlu receptor agonist showed beneficial effects in a Phase II clinical trial, with significant improvement in both positive and negative symptoms compared to placebo in chronic schizophrenia patients. However, there was no significant difference in any of the measures compared to olanzapine. In a subsequent Phase II trial, neither LY2140023 nor olanzapine showed any significant difference compared to placebo, with a very high placebo response observed in this trial and thus this study was ruled inconclusive (Kinon *et al*, 2011). N-acetyl cysteine (NAC), a glutathione precursor has shown promising results in terms of primarily improving negative symptoms in chronic schizophrenia patients (Berk *et al*, 2008). However, another study with NAC did not find any effect on negative symptoms but instead reported an improvement in positive symptoms in schizophrenia, particularly in patients with a longer duration of illness (Rapado-Castro *et al*, 2015). A large scale clinical trial is currently underway investigating the effects of NAC treatment in patients with schizophrenia resistant to clozapine (Rossell *et al*, 2016). Ideally, in order for new drugs to be developed and successfully tested, it would be beneficial to be able to determine whether these drugs are achieving their desired physiological effects, before trying to test their clinical efficacy. MRS can be used to study neurochemical concentrations pre and post administration of a drug, and can provide extremely valuable information to aid drug development prior to large scale clinical trials.

Ultimately, the goal of translational neuroimaging research is to provide clinically valuable information in order to aid diagnosis, prognosis and treatment. Although precision medicine in schizophrenia and psychiatry remains a challenge, recent scientific advances in many areas of research

including neuroimaging methods development, imaging genetics and alternate treatments for schizophrenia such as repetitive Transcranial Magnetic Stimulation (rTMS) are bringing us closer to this goal.

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