An investigation of the link between cortical inhibition, neural oscillations and psychophysics in schizophrenia

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Summary

Schizophrenia is a highly complex psychiatric disorder with a lifetime risk of approximately 0.4-0.7%. Alterations in the major inhibitory neurotransmitter GABA have been identified in the brains of those with schizophrenia. Studies examining the nature of these differences have given variable results depending on the type of patient group, medication and the brain region where *in vivo* MRS GABA was measured. Chapter 5 of this thesis utilises the non-invasive imaging tool magnetic resonance spectroscopy (MRS) to investigate differences in GABA levels in two distinct areas of the brain of those with schizophrenia, whilst adjusting for important potential confounds such as antipsychotic medication dosage.

GABA is also of interest due to its link with synchronised oscillatory activity, primarily gamma activity that is implicated in connectivity between different brain regions as well as cognitive functioning, thus demonstrating its potential relevance to schizophrenia research. Chapter 6 investigates differences in gamma activity between those with schizophrenia and controls induced by a static stimulus and a moving radial stimulus, both known to induce strong visual gamma responses, using magnetoencephalography (MEG) imaging methods.

Because GABA and gamma measures are robust for visual cortex measures, we used visual psychophysics tasks thought to be dependent on inhibitory processes - orientation discrimination and the tilt illusion, to see if there is a behavioural deficit in schizophrenia and if this also relates to changes in GABA and gamma measures. Chapters 3 and 4 set out to validate the psychophysics and to establish whether they were appropriate paradigms for the patient group for which they were intended. Chapter 7 brings together the imaging and psychophysics to see if a relationship exists between them in both the patient and control groups.

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Glossary

The following abbreviations are used throughout this thesis:

AMPA - Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

GABA – Gamma-amino butyric acid

GAD – Glutamic acid decarboxylase

MEG – Magnetoencephalography

MRI – Magnetic resonance imaging

MRS – Magnetic resonance spectroscopy

OD – Orientation discrimination

PO – Preferred orientation

PSE – Perceived subjectivity equality

SAM – Synthetic Aperture Magnetometry

TI – Tilt illusion

Contents

Chapter 1 - Introduction

1.1 Rationale

Schizophrenia is a highly complex psychiatric disorder with a lifetime risk of approximately 0.4-0.7% and is currently the seventh most costly disorder to society. It is split into three symptom categories; positive symptoms which include perceptual hallucinations and delusions, negative symptoms including emotional blunting, negative thought disorder and social withdrawal and finally cognitive deficits which span all cognitive domains.

Environmental and genetic factors appear to play a role in the development of the disorder and so pinpointing a target for treatment development has not been easy. Current treatments focus on the dopamine system and whilst they treat the positive symptoms effectively, they do little to reduce the negative and cognitive symptoms, arguably the most debilitating. Therefore new treatment targets are needed to treat these additional symptoms.

The GABAergic system first became a candidate target for treatment development due to human post-mortem studies showing a reduction in the GABA synthesizing enzyme GAD-67 in the brains of patients with schizophrenia. Recent genetic research has also supported the idea of GABA being implicated in this disorder.

GABA is also of interest due to its link with synchronised oscillatory activity, primarily gamma activity. Gamma oscillations are implicated in connectivity between different brain regions and cognitive functioning so are of relevance to schizophrenia research. *In vivo* measurement of GABA in patients has

produced mixed and inconsistent results but in chronic patients reductions are shown in differing brain regions. Gamma activity in patients is also shown to be reduced in patients.

It is important to relate the imaging measures to behaviour in both healthy and disease populations. Behavioural tasks can then be used as additional markers in relation to the imaging measures and be used as proxy measures in clinical settings. This thesis will therefore investigate if there are between group differences in measures of *in vivo* MRS GABA and gamma activity measured using MEG methods. It will also incorporate psychophysics to test whether there is a relationship between imaging and behavioural measures in the hope of developing these into possible biomarkers of schizophrenia.

1.2 Schizophrenia

Schizophrenia is a highly heritable psychiatric disorder with a lifetime risk of approximately 0.4-0.7% (Prince, Patel, Saxena, Maj, Maselko, Philips et al., 2007). It is a neurodevelopmental disorder characterised by three major symptom groups. Firstly positive symptoms, which include delusions and perceptual hallucinations, secondly negative symptoms including emotional blunting, lack of motivation and paucity of speech, and lastly marked cognitive impairment typically across cognitive domains. A diagnosis of schizophrenia can encompass different combinations from these symptom groups but there has to be a severe impairment to the patient's daily life in areas such as employment and social functioning for more than 6 months for a diagnosis to be given based on the Diagnostic and Statistical Manual of Mental Disorders Version 5 (DSM-V) criteria (American Psychiatric Association, 2013).

Schizophrenia is a complex disorder with differences in gender distribution and women more likely to experience onset of symptoms in their late twenties/early thirties compared to late teens/early twenties for men (Goldstein, 1997). Women are also more likely to have an affected family member and to experience more positive and affective symptoms, whereas men tend to experience more negative symptoms and have poorer long-term outcomes (Goldstein, 1997; Ochoa, Usall, Cobo, Labad, and Kulkarni 2012).

Whilst some patients can experience one episode of psychosis and recover well for the rest of their lives, for others the symptoms associated with schizophrenia can be highly debilitating for both the person affected and family members, with 50% of patients attempting suicide and 10% succeeding (Meltzer and Fatemi, 1995). Furthermore, it is estimated that only 30% of people diagnosed are classed as having a positive outcome with no long-lasting impairment after an acute psychotic episode, with another 30% experiencing persistent, progressive symptoms causing increasing impairment and most likely permanent hospitalisation (Ross, Margolis, Reading, Pletnikov and Coyle, 2006). Due to the early onset of the disorder and its frequently chronic debilitating nature, it is the seventh most costly disorder to society due to hospitalisation costs, lack of employment and medication (Pearlson, 2000). It is thus evident why research has and is still focusing on the aetiology and risk factors associated with this disorder.

1.3 Risk factors

1.3.1 Environmental

Several environmental risk factors have been identified relating to the development of schizophrenia. They fall into the following categories: place/time of birth, infection, prenatal complications and obstetric complications (Sullivan, 2005; see Figure 1). As demonstrated in Figure 1, the largest risks are related to maternal health during pregnancy, including contracting influenza in the 2nd semester, suffering a bereavement during pregnancy or obstetric complications resulting in central nervous system damage to the child. All of these can increase the risk of the child developing schizophrenia by at least 5 times that of the healthy population (Sullivan, 2005; see Figure 1.1). The issue of maternal influenza could be linked to the increased risk of developing schizophrenia if birth occurs in the winter months and in an urban population, as there is an increased likelihood of contracting flu during these months especially in densely populated areas. Cannabis use also infers an approximately two-fold increase in the likelihood of developing schizophrenia, although it does not appear to be necessary or sufficient in the development of the disorder, more it's part of a number of factors than can lead to psychosis (Arseneault, Cannon, Witton and Murray, 2004; Arendt, Rosenberg, Foldager, Perto, and Munk-Jørgensen, 2005). However, the main risk factor highlighted in Figure 1.1 is family history. This strongly suggests there is a genetic component to the disorder.

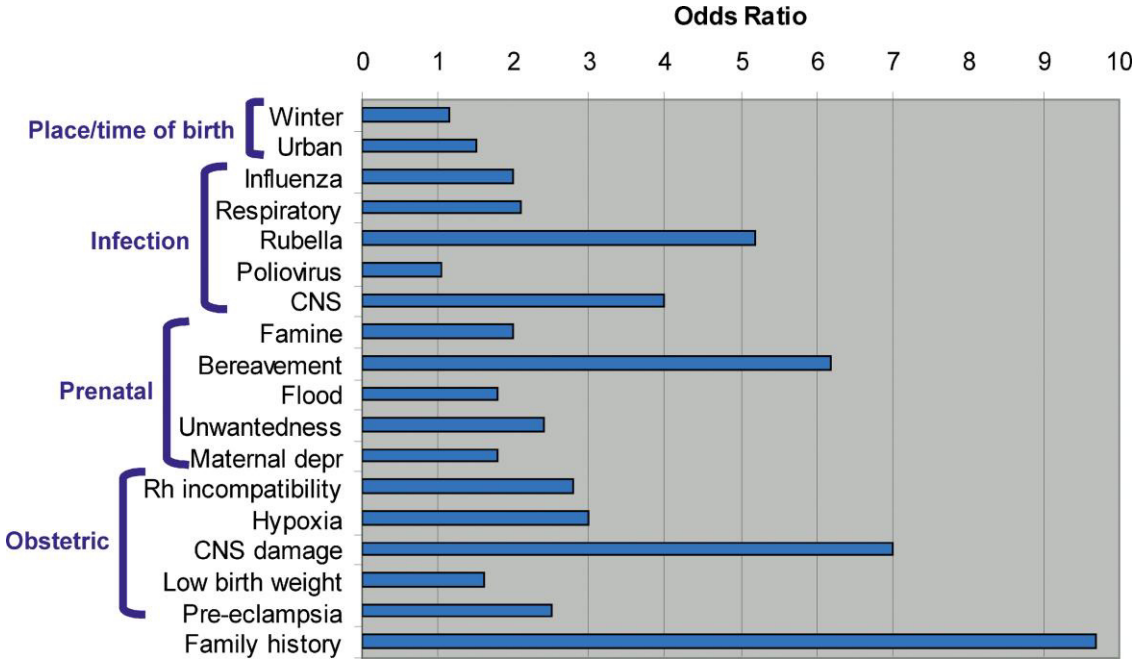


Figure 1.1. Environmental risk factors associated with schizophrenia (taken from Sullivan, 2005)

1.3.2 Genetic risk

Evidence for a strong genetic component to the development of schizophrenia is shown by the high concordance rate amongst first-degree relatives of a person with a diagnosis of schizophrenia. For example estimates of the concordance for schizophrenia in monozygotic (MZ) twins are 40-70%, and 17% for dizygotic (DZ) twins (Gottesman, 1991 see figure 1.2; Pearlson, 2000; Sullivan, 2005). Thus, the risk of developing schizophrenia increases with the degree of genetic relatedness to the proband (the person with the diagnosis). However, with a maximum 70% concordance in MZ twins, these results also point to the importance of environmental factors.

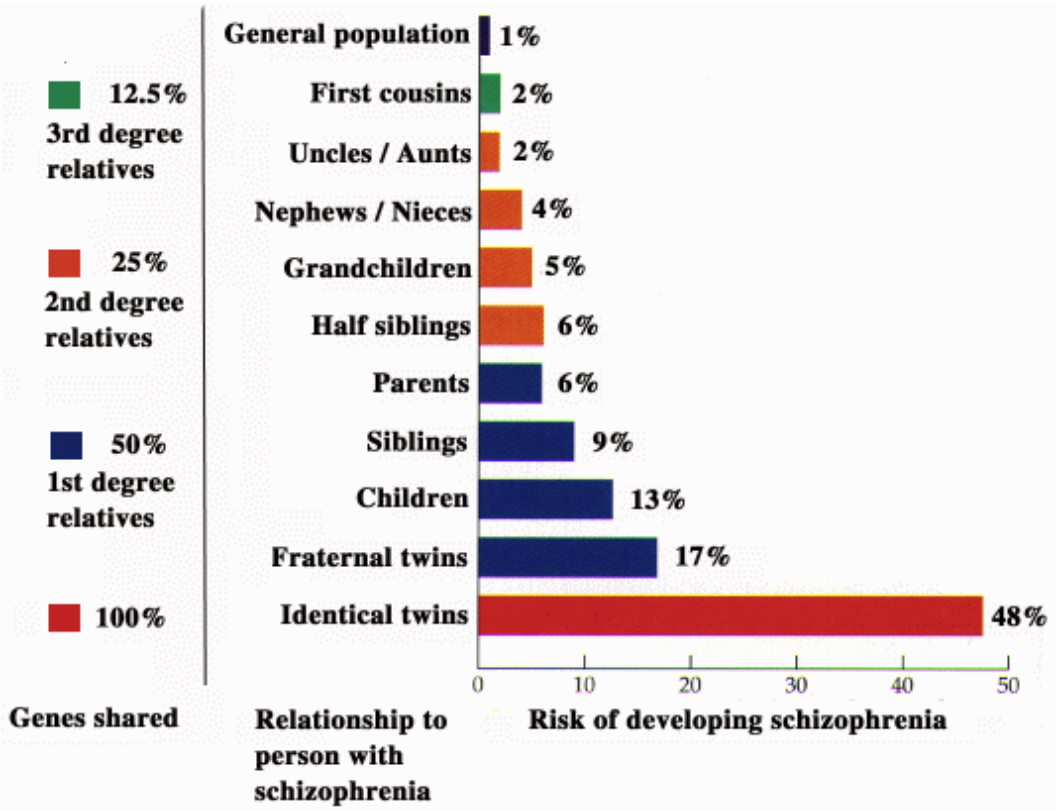


Figure 1.2. Genetic risk of schizophrenia (taken from Gottesman, 1991)

Unfortunately, unlike other disorders such as Huntington's disease, research has not been able to identify a definitive association of a single gene variant with the development of schizophrenia (Ross et al., 2006). Instead it would appear that there is a polygenic risk picture of multiple genetic variants that confer a small level of risk, which can combine to produce a cumulative risk for development of the disorder (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). In line with the odds ratios of environmental factors, the involvement of these susceptibility genes for schizophrenia can at times only account for an increase in the risk of as little as 1.1 to 3 times. In recent years there has been the discovery of large (>1 megabase), rare (<1%), duplications or deletions of chromosomal regions found to increase the risk of developing schizophrenia. These copy number variants are associated with

odds ratios between 2 and over 30, so confer substantial increased risk - though collectively are seen in only around 2.5% of those with the disorder (Rees, Walters, Georgieva, Isles, Chambert, Richards et al, 2014).

The development of the disorder is now thought of as a combination of a genetic predisposition i.e. carrying risk genetic variants, which then interacts with environmental factors during early brain development causing possible structural or neurochemical abnormalities (Tsuang, 2000; Owen, Craddock, O'Donovan, 2005).

1.4 Aetiological theories

1.4.1 The Dopamine hypothesis

Neurotransmitter dysregulation, especially within the dopaminergic system, as a possible mechanism for the development of schizophrenia, and particularly psychosis, has been one of the most popular and lasting theories regarding this illness. This hypothesis arose from the observation that administration of amphetamines, dopamine agonists that increase the level of dopamine in the synapse, induces psychotic-like symptoms in healthy controls (Randrup and Munkvad, 1967) and exacerbates existing symptoms in patients (Crayton, Meltzer and Goode, 1968; Snyder, Banerjee, Yamamura and Greenberg, 1974). Particular attention has been paid to the dopamine D2 receptor, as it emerged that D2 receptor blockade, which reduced dopamine binding at the receptor, was the mechanism behind prescribed anti-psychotics (Seeman, Chau-Wong, Tedesco and Wong, 1975). Further, in first episode patients, the density of D2 is elevated by ~10% to 30% in the frontal cortex (Kessler, Ansari, Riccardi, Li, Jayathilake, Dawant and Metzler 2006) and striatum (Wong,

Pearlson, Tune, Young, Meltzer, Dannals et al 1997). Hyperdopaminergia was therefore the focus of this theory of the development of schizophrenia.

1.4.2 Challenges to the dopamine hypothesis

All current licensed antipsychotic drugs work by blocking D2 receptors. The number of D2 receptors also predicts the speed and the nature of a subject's response to the antipsychotic (Abi-Dargham, Rodenhiser, Printz, Zea-Ponce, Gil, Kegeles et al, 2000). These antipsychotics, however, are most effective at treating the positive symptoms but do not impact cognitive deficits and negative symptoms experienced by those with schizophrenia. This is still a major obstacle to the treatment of this disorder and the reduction of these cognitive deficits and negative symptoms is, therefore, a new treatment target.

A further problem arises when one considers that the administration of anti-psychotics that block dopamine receptors does not ameliorate psychotic symptoms in all cases, with as many as 30% of patients relapsing whilst taking anti-psychotics (Harding and Hall, 1997). Additionally, of those who do respond well, the vast majority still experience symptoms In addition it has been demonstrated that not all patients experienced heightened symptoms when administered amphetamines, with some patients actually reporting an improvement in their symptoms (Van Kammen and Boronow, 1988; Dickson, Allen and Werner, 1995). This suggested that there is a wide-ranging diversity in the pathophysiology of schizophrenia and that additional biological mechanisms should be considered.

1.4.3 GABA and Glutamate

GABA is the major inhibitory neurotransmitter in the mammalian brain. It is synthesised by the process of decarboxylation of glutamate, the major excitatory neurotransmitter in the brain, via the enzyme glutamate decarboxylase (GAD; Olsen and DeLorey, 1999). Some of the principal pathways of the GABA/glutamate cycle are schematised in Figure 1.3. Calcium-dependent release of GABA results in an increase of GABA concentration within the synapse and therefore an increase in receptor binding at the post-synaptic neuron, in this case on the GABAa receptor site as this is the active site for GABA binding. The difference between the two types of GABA receptors are that GABAa receptors are fast acting whereas GABAb receptors are slow acting and have been linked to processes such as behavioural aspects of ethanol (Dzitoyeva, Dimitrijevic, & Manev, 2003) and pain response (Manev and Dimitrijevic, 2004). Removal of GABA occurs rapidly by way of the GABA transporter (GAT) via reuptake processes into the presynaptic neuron and/or surrounding glia cells. GABA is then broken down into metabolites, which are subsequently used in the resynthesis of GABA via glutamate and glutamine.

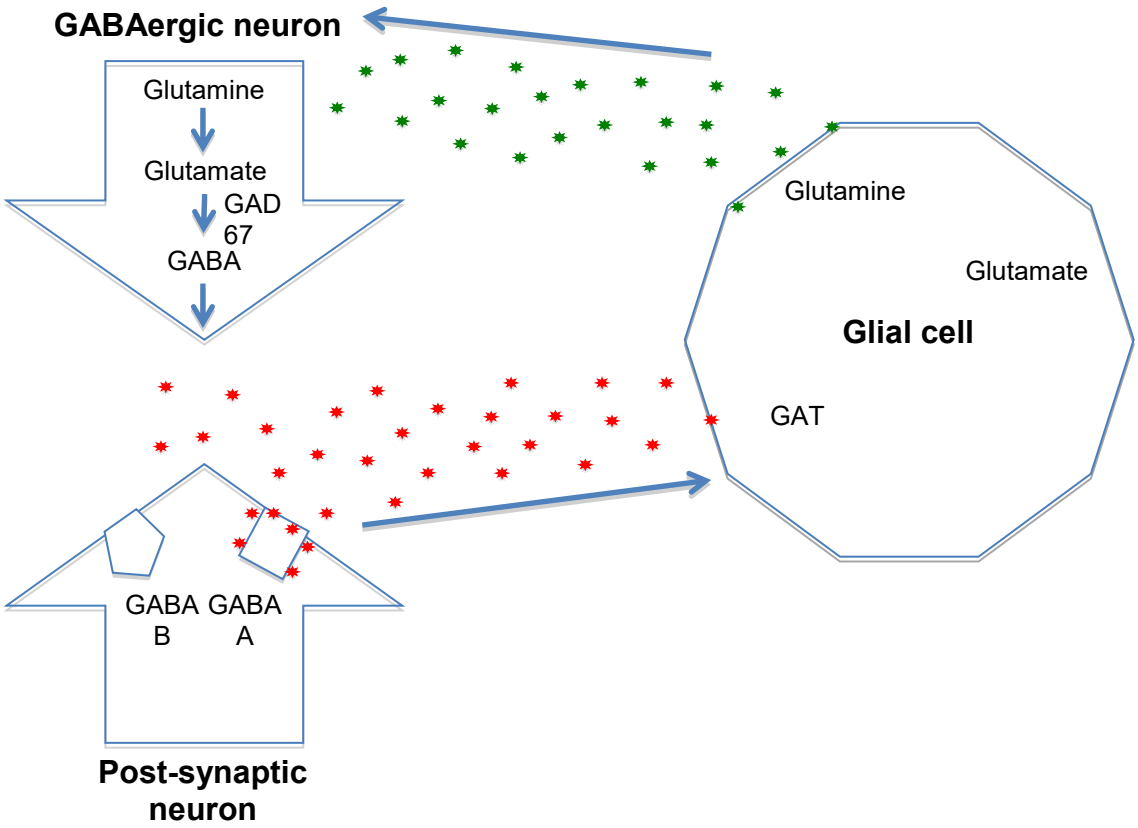


Figure 1.3. Red dots represent GABA synthesis and green dots represent glutamine to glutamate synthesis.

Cortical neural circuits are comprised of both excitatory neurons, mostly glutamatergic pyramidal cells, and inhibitory neurons, mostly GABAergic parvalbumin containing (PV+) neurons. It is these PV+ neurons that are of interest as it is widely accepted that inhibition arising from this sub-type of neurons is essential for synchronisation of neural activity (Bartos, Vida and Jonas, 2007; Gonzalez-Burgos, Hashimoto and Lewis, 2010).

1.4.4 The GABA hypothesis

A gamma-amino-butyric-acid (GABA) disturbance in cases of schizophrenia was first suggested by Roberts (1972). It was hypothesised that the symptoms associated with schizophrenia could be accounted for by low levels of GABA in the brain leading to a state of disinhibition. Since this theory was postulated,

there have been many studies investigating the link between GABA and dopamine. Application of the GABA agonist muscimol inhibited the firing of dopamine neurons in both the substantia nigra and the ventral tegmental area, where dopamine tracts are thought to be overactive in schizophrenia. Thus leading to an increase in GABA and reduction in downstream dopamine. Figure 1.4 depicts the opposite direction of effect with the introduction of a benzodiazepine (GABA antagonist) reducing GABA levels and increasing dopamine levels downstream. This finding therefore closely relates to the idea of a reduction in GABA and hyperdopaminergia in schizophrenia.

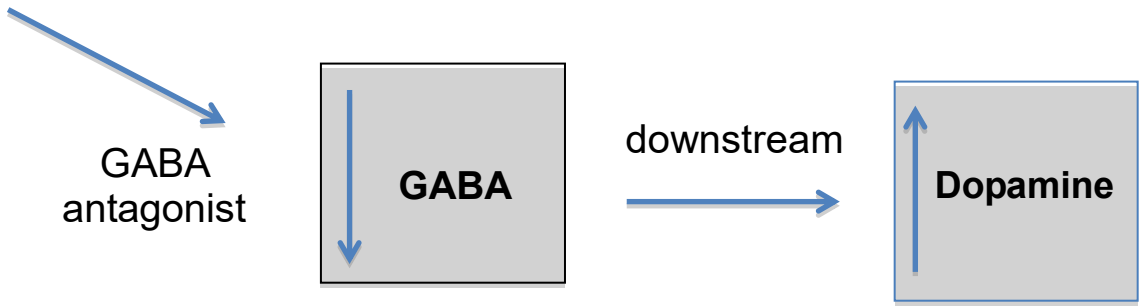


Figure 1.4. The effect of GABA antagonists on GABA and dopamine levels indicated by arrows.

1.4.5 GABAergic theory of schizophrenia

The idea of a reduction in GABA levels or GABAergic properties in patients with schizophrenia is widely supported. The most consistently reported finding is a reduction in the 67-kDa isoform of the GABA synthesising enzyme glutamic acid decarboxylase (GAD67); (Curley, Arion, Volk, Asafu-Adjei, Sampson, Fish et al 2011; Guidotti, Auta, Davis, Gerevini, Dwivedi, Grayson et al, 2000), which is thought to synthesise ~70% of brain GABA (Soghomonian and Martin, 1998). Figure 1.5 illustrates the reductions in inhibitory basket cells and how this relates to excitatory pyramidal cells. Work with *GAD67* knockout mice (i.e. mice

that express no GAD67) supported these findings by showing that these mice produced only 7% of the GABA concentrations produced by wild-type mice (Asada, Kawamura, Maruyama, Kume, Ding, Kanbara et al, 1997). This strongly suggested that a reduction in the expression of this enzyme would lead to a reduction in GABA levels overall. In addition to this finding, a single-nucleotide-polymorphism (SNP) in the *GAD1* gene, which encodes for GAD67, is associated with reduced mRNA expression in the prefrontal cortex of patients with schizophrenia (Straub, Lipska, Egan, Goldberg, Callicott, Mayhew et al, 2007).

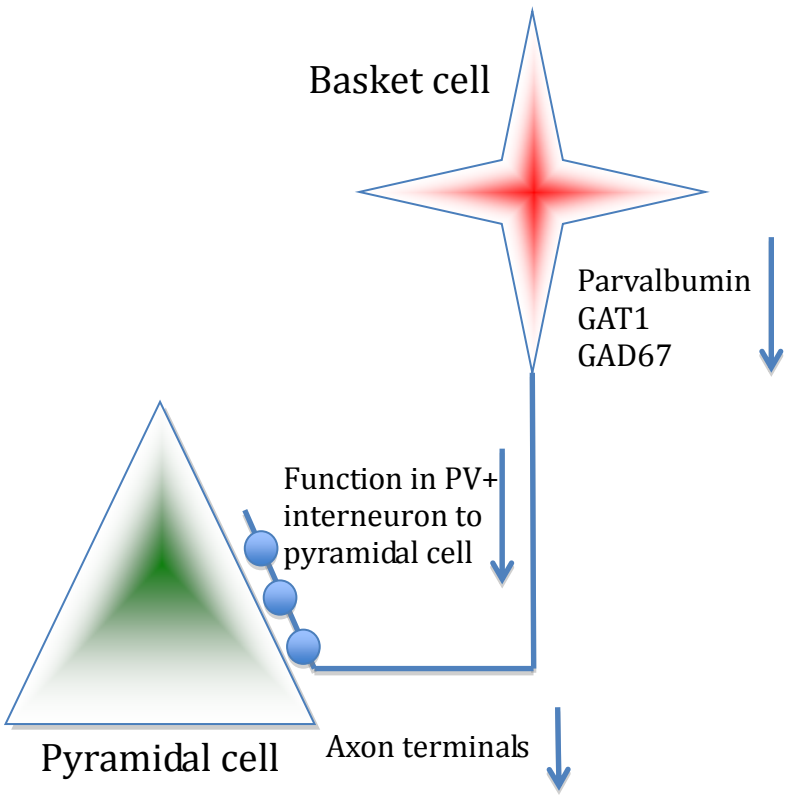


Figure 1.5. Alterations found in cortical circuits in patients with schizophrenia. Arrows indicate the direction of effect.

A further compelling finding is the reduction in expression of neuregulin-1, a tropic factor important for brain development and encoded by the schizophrenia

susceptibility gene *NRG1* (Munafo, Thiselton, Clark and Flint, 2006). PV+ neuron signalling is strongly mediated by neuregulin-1 with one of its receptors, ErbB4, being enriched in GABA neurons and in particular PV+ neurons. Fisahn, Neddens, Yan and Buonanno (2009) demonstrated how expression of NRG-1 increased gamma power, neural oscillations that occur at a frequency of 30-150Hz, in the hippocampus with these effects blocked by a pan-specific ErbB receptor antagonist. This showed that gamma power is mediated by ErbB4 receptors as mice bred with a mutation of ErbB4 did not respond to increased expression of NRG-1.

Recent advances in genetics research mean that Genome-Wide Association Studies (GWAS) has discovered a new class of genetic variation, large deletions and duplications of DNA called copy number variants (CNVs). CNVs are very rare and work utilising GWAS chip methods has discovered that a small number CNVs are associated with the disorder (Walsh, McClellan, McCarthy, Addington, Pearce, Cooper et al, 2008; Need, Ge, Weale, Maia, Feng, Heinzen et al, 2009). These are alterations of the DNA that results cells having an abnormal number of copies of one or more sections of the DNA. CNVs within one gene in particular, neurexin-1, have been confidently identified (Rujescu, Ingason, Cichon, Pietiläinen, Barnes Toulopoulou, et al., 2009) with several others, spanning many different genes, also imparting risk (Rees et al., 2014). Because of the different genes involved it has been hard to pinpoint the mechanism by which they contributed to the development of the disease. However, Pocklington, Rees, Walters, Han, Kavanagh, Chambert et al (2015) have, for the first time demonstrated, using over 11,000 cases, that CNVs are enriched for genes involved in GABAergic transmission, most of which relate to

GABAa receptor complexes. This shows that GABAergic signaling is of causal relevance to the disorder. In addition they also replicated previously found enrichment of CNVs for glutamatergic transmission. This lends further support to the idea that an imbalance of the inhibitory/excitatory activity of neurons contributes to schizophrenia.

It is important to determine the processes that underlie these irregularities in cortical inhibition in humans using in vivo methods. Whilst animal studies/models are extremely useful, the methods used are invasive and it is not certain if the results are directly transferable to humans and their behaviour. Biomarker development is now a key aim of research into disease as these can be used as a non-invasive indicator of a particular physiological state. Imaging biomarkers can and have been extremely useful in many different areas including cancer detection using MRI spectroscopy methods (Fischer, Kettunen, Würtz, Haller, Havulinna, Kangas, et al, 2014), the identification and treatment of heart disease (Caplan, Waxman Nesto and Muller, 2006; Wang, Balu, Canton and Yuan, 2010) and) and potentially prediction of responsiveness to treatment using EEG in schizophrenia patients (Khodayari-Rostamabad, Hasey, MacCrimmon, Reilly and de Bruin, 2010) although this does need replication studies. These examples demonstrate a number of ways biomarkers can help: identification of a disease, predicting responsiveness of treatment and monitoring the effects of treatments. All of these are needed for the diagnosis and management of schizophrenia. I will now provide a summary of relevant in vivo imaging studies related to schizophrenia.

1.5 Imaging studies of schizophrenia

For the purpose of this introduction only a very brief overview of each imaging method will be provided. Relevant approaches will be discussed more fully in Chapter 2.

1.5.1 Magnetic Resonance Imaging (MRI)

Structural differences in the brains of patients with schizophrenia are well established. Early encephalogram and computerised tomographic (CT) studies found significantly enlarged ventricles in patients compared to controls (Haug, 1962; Johnstone, Frith, Crow, Husband and Kreel, 1976). Post-mortem studies have also verified this finding (Pakkenberg, 1987). Pakkenberg also identified whole brain volume, hemisphere volume, the cortex and gray matter volume were all reduced in patients compared to controls. These findings have been widely replicated with the reduction in volume and tissue content, predominantly gray matter, appearing to be present in different brain regions including the thalamus, caudate nucleus and prefrontal cortex (Haijma, Van Haren, Cahn, Koolschijn, Hulshoff Pol and Kahn (2013), the dorsolateral prefrontal cortex, inferior parietal lobe and superior temporal gyrus (Peng, Lee, Federman, Chase, Barta and Pearlson, 1994) and the hippocampus and amygdala (Velakoulis, Wood, Wong, McGorry, Yung, Phillips et al, 2006; van Erp, Hibar, Rasmussen,Glahn, Pearlson, Andreassen et al., 2015).

1.5.2 Functional MRI

Functional MRI (fMRI) is a method used to infer brain activity by measuring the associated changes in blood oxygenation level (Huettel, Song and McCarthy,

2004). Brain activity is assessed through a proposed indirect haemodynamic correlate, specifically the difference in magnetic properties of oxygenated and deoxygenated blood leading to differences in the MR signal. Neuronal firing causes a need for more energy to be brought in quickly. Through a process called the haemodynamic response, blood releases oxygen to them at a greater rate than to neurons not firing. This causes a change of the relative levels of oxygenated and deoxygenated blood that can be detected by the MRI (Ogawa, Lee, Kay and Tank (1990).

Within patient groups abnormalities within resting-state networks, as evidenced by reduction in spontaneous BOLD signals, have been reported (Bluhm, Miller, Lanius, Osuch, Boksman, Neufeld et al, 2007; Woodward, Rogers and Heckers, 2011), as have reductions in the BOLD signal during cognitive tasks (Rasser, Csernansky, Gado and Barch, 2014).

However, there are limitations with using fMRI. Firstly it has not been determined whether the BOLD response truly relates to activity or if it is just a proxy measure. Secondly the magnitude of the BOLD response has been linked to physiological parameters such as cardiovascular fitness; the fitter you are the stronger the BOLD signal (Gonzales, Tarumi, Mumford, Ellis, Hungate, Pyron et al, 2014). Obesity is a major issue within patients with schizophrenia due to sedentary lifestyle, unhealthy eating habits, the illness itself and medication (Hjorth, Davidsen, Hilian and Skrubbeltrang, 2014). Therefore, the use of other imaging methods to determine levels of neural activity would be more appropriate to this patient group.

1.5.3 Magnetic resonance spectroscopy (MRS)

MRS is an MRI technique that allows the non-invasive in vivo measurement of concentrations of different metabolites within the brain, including creatine, alanine, choline, N-Acetyl Asparate (NAA), glutamate/glutamine (Glx) and GABA (Jansen, Backes, Nicolay and Kooi, 2006).

A reduction in NAA has been reported and replicated throughout the brain in patients with schizophrenia (Rowland, Bustillo, Lauriello, 2001), as has a reduction in glutamate levels coupled with an increase in glutamine (Marsman, van den Heuvel, Klomp, Kahn, Luijten and Pol, 2013) and creatine (Öngür, Prescot, Jensen, Cohen, Renshaw, 2009). However, many of these studies do not have enough power to reliably detect a difference in metabolites between groups and so these findings may not be as reliable as first thought (Steen, Hamer and Lieberman, 2005)

These metabolites can be difficult to measure using a 3 Tesla MRI scanner due to poor signal-to-noise and relatively broad spectral line-widths for many of the desired molecules. GABA, however, can be measured using a scanner of this strength when using the PRESS method making it a reliable metabolite for research (Mullins, Chen, Xu, Caprihan and Gasparovic, 2008)

When looking at GABA levels in vivo, results for patients are mixed, with some reporting an increase in GABA in chronic patients (Öngür, Prescot, McCarthy, Cohen & Renshaw, 2010) while others report a reduction (Yoon, Maddock, Rokem, Silver, Minzenberg, Ragland and Carter, 2010; Marsman, Mandl, Klomp, Bohlken, Boer & Andreychenko et al, 2014). One possible reason for

these differences is the level and variety of antipsychotic medication being taken by patients. Whilst evidence from clinical studies suggests that antipsychotic medication could affect the GABAergic system, either at the receptor level or by altering GABA concentrations (Wassef, Baker & Kochan, 2003), in vivo results however, are mixed. Tayoshi, Nakataki , Sumitani, Taniguchi, Shibuya-Tayoshi, Numata et al (2010) reported a significant difference in GABA levels between patients taking typical versus atypical antipsychotics suggesting the type of antipsychotic could be a factor. Administration of clozapine has been associated with an increase in GABA-B transmission (Kaster, Jesus, Radhu, Farzan, Blumberger, Rijji et al, 2015) whereas Rowland, Krause, Wijtenburg, McMahon, Chiappelli, Nugent et al (2015) demonstrated that after converting antipsychotic medications to their chlorpromazine equivalents, and when included as a covariate in their analysis, it did not change their finding of a reduction in GABA levels in patients with schizophrenia. These findings are very mixed and it is difficult to compare groups, as they are quite different in terms of length of illness and studies usually only account for current and not lifetime medication use. This could be very important when standardising medications to add as a covariate because lifetime exposure could also affect GABA levels.

The brain region where GABA is measured can produce different results. Although post-mortem studies suggest that GAD67 expression is reduced throughout the brain in patients. Kegeles, Mao, Stanford, Girgis, Ojeil, Xu et al (2012) found conflicting results from two voxels positioned in the dorsolateral and medial prefrontal cortex, with an increase in GABA levels in unmedicated patients in the medial prefrontal region but not in the dorsolateral prefrontal

cortex. This study also reported no differences in GABA levels between healthy controls and medicated patients in both voxels, suggesting medication within these brain regions, may reduce GABA in patients. Marsman et al., (2014) also found reductions in GABA, in relation to creatine, in the prefrontal cortex of patients compared to controls, but found no difference in parietal-occipital cortices. These findings again suggest that the differences found in GABA levels in patients are highly variable across studies and are not consistent throughout the brain. These changes appear to be most common in the prefrontal cortex, which could potentially account for the deficits in high-level functioning experienced by patients. More research investigating GABA levels in different areas of the brain is needed to determine the true extent of this reduction.

1.5.4 Neural oscillations

Neural oscillations are the repetitive or rhythmic firing of neurons within the brain, which results in the production of oscillations. These can be measured using either electroencephalography (EEG) or magnetoencephalography (MEG) measures. MEG is a non-invasive imaging technique used to measure activity generated in the brain. It measures the synchronised firing of tens of thousands of neurons and maps this activity back to its source within the brain. Outputs from such data include, but are not limited to, transient amplitude and frequency, which is the initial spike in activity generated by the presentation of a stimulus, and sustained amplitude and frequency which is the sustained level of activity for a specified amount of time after stimulus onset. The frequency measures at which these can be measured are as follows; delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (8-30Hz) and gamma (30-150Hz). Gamma

oscillations are of particular interest as they have been implicated in cognition and connectivity between different brain regions (Montgomery and Buzsaki, 2007; Colgin, Denninger, Fyhn, Hafting, Bonnevie, Jensen et al, 2009). In addition, they are known to be the product of interplay between GABA and glutamatergic principal cells (Traub, Bibbig, LeBeau, Buhl and Whittington, 2004). Therefore this thesis will focus only on gamma band oscillations.

1.5.5 Gamma oscillations

Gamma (γ) oscillations occur at a frequency of between 30-150Hz and are heavily implicated in information transfer between different brain regions and with the idea of 'connectedness' within the brain (Montgomery and Buzsaki, 2007; Colgin, et al, 2009). They are of particular interest due to their link with synaptic inhibition with GABA-mediated inhibition from PV+ neurons heavily implicated in their generation (Gonzalez-Burgos and Lewis, 2008; Sohal, Zhang, Yizhar, and Deisseroth, 2009).

PV+ neurons provide strong inhibition onto excitatory pyramidal cells and other GABAergic neurons, including other PV+ neurons. Computational models have shown how mutual inhibition between PV+ neurons is essential for the generation of γ oscillations (Bartos et al., 2007) but in studies using genetically modified mice with GABAa receptor mediated inhibition removed from PV+ cells, the amplitude and frequency of γ activity was the same suggesting mutual inhibition is not necessary for γ activity (Wulff, Ponomarenko, Bartos, Korotkova, Fuchs, Bähner, et al., 2009)

Neuron excitation, however, does appear to be necessary for the generation of γ activity and in computational models glutamatergic pyramidal cells provide excitation, which are in turn inhibited in a feedback loop. Recent work has identified the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype of glutamate receptors as being necessary to provide normal oscillations as mice bred with a deletion of AMPA receptor subunits GluR-D or GluR-D in PV+ neurons have reduced power compared to wild-type mice (Fuchs, Zivkovic, Cunningham, Middleton, LeBeau, Bannerman et al, 2007).

However, in support of the role of inhibition in normal activity, the reduction in GAD67 expression discussed previously has been localised to PV+ GABAergic interneurons, which has been confirmed in post-mortem studies in the motor and visual cortex (Hashimoto, Bazmi, Mirnics, Wu, Sampson & Lewis, 2008), the hippocampus (Knable, Barci, Webster, Meador-Woodruff ,Torrey and Stanley Neuropathology Consortium, 2004) as well as the dorsolateral prefrontal cortex (Akbarian, Kim, Potkin, Hagman, Tafazzoli, Bunney et al, 1995). This finding suggests that the reduction in synthesis of GABA is specific, though not exclusive, to the sub-population of neurons necessary for the production of oscillations.

1.5.6 Visually-induced gamma

Induced activity refers to activity generated in the brain that is non-phase locked to the stimulus presented (Galambos, 1992). It is also referred to as a self-generated activity (Uhlhaas and Singer, 2010). This is compared to evoked responses that are phase-locked to the stimulus.

Visually-induced gamma activity has now become one of the most studied areas in relation to neural oscillations largely because simple high contrast static grating stimuli have been shown to elicit consistent and repeatable occipital **γ** -band oscillations in healthy controls (Muthukumaraswamy, Singh, Swettenham & Jones, 2010). Annulus grating stimuli and larger full screen stimuli has been shown to increase gamma power compared to square-wave grating stimuli (Muthukumaraswamy and Singh, 2013). A reduction in amplitude is also seen when using a plaid grating stimulus (Hermes, Miller, Wandell & Winawer, 2014). Moving grating stimuli have been shown to increase both amplitude and frequency, whilst additionally producing results with a clearer induced sustained effect in healthy controls at approximately 65-70Hz (Swettenham, Muthukumaraswamy and Singh, 2009; Muthukumaraswamy and Singh, 2013).

An attentionally engaging stimulus also produces an increase in amma frequency (Vidal, Chaumon, O'Regan, & Tallon-Baudry, 2006) and, when used in conjunction with contracting radial moving stimuli, produces similar results with a clear sustained effect between 65-70Hz as well as an additional separate amma band at around 40Hz for a subset of participants (Hoogenboom, Schoffelen, Oostenveld, Parkes & Fries, 2009).

Gamma oscillations are related to cognition and the ideas of ‘connectedness’ within the brain (Montgomery and Buzsaki, 2007; Colgin, et al, 2009) and so have become a prime area of research for psychiatric disorders. A reduction in gamma oscillatory activity has been widely reported in patients with schizophrenia with recent work showing a general reduction in induced gamma amplitude both at rest and during working memory tasks (Haenschel, Bittner,

Waltz, Haertling, Wibral, Singer et al, 2009; Chen, Stanford, Mao, Abi-Dargham, Shungu, Lisanby et al, 2014) and backward-masking tasks (Wynn, Light, Breitmeyer, Nuechterlein & Green, 2014). Additional reductions in high gamma power (60-120Hz) have been recorded during perception of Mooney faces (Grutzner, Wibral, Sun, Rivolta, Singer, Maurer et al, 2013), auditory stimulation (Hamm, Gilmore, Picchetti, Sponheim & Clementz, 2011) and perceptual organisation (Tillmann, Wibral, Leweke, Kohler, Singer, Koethe et al (2008).

Antipsychotic medication has been shown to modulate gamma activity irrespective of disease state. Rats under the influence of both first and second-generation antipsychotics showed inhibited hippocampal gamma power due to the medication's interaction with D3 dopamine receptors (Schulz, Heidmann, Mike, Klaft, Heinemann & Gerevich, 2012). However, the same pattern of results have been found irrespective of medication state as unmedicated first episode patients have demonstrated impaired cognitive control related gamma activity and also reduced evoked prefrontal gamma-band responses during an auditory task (Minzenberg, Firl, Yoon, Gomes, Reinking & Carter, 2010; Gallinat, Winterer, Herrmann & Senkowski, 2004 respectively). It could be the case that both antipsychotic medication and the disorder itself independently affect oscillatory mechanisms and that the combination amplifies the effect. Brain structure has been implicated as a mediating factor in the disruption of neural oscillations and has been related to gamma frequency with the surface areas of V1 and V2 positively correlating with visually induced gamma in healthy controls (Schwarzkopf, Robertson, Song, Barnes, & Rees, 2012). Cortical thickness in area V1, peak transient and peak sustained gamma frequency have been negatively correlated with age (Gaetz, Roberts, Singh,

Muthukumaraswamy, 2011). Reductions in cortical volume, thickness and surface area in patients have also been reported (Rimol, Nesvåg, Hagler Jr, Bergmann, Fennema-Notestine, Hartberg et al, 2011; Edgar, Chen, Lanza, Howell, Chow, Heiken et al (2014). Any investigation of how modulations of gamma oscillatory dynamics relates to behaviour and/or disease state needs to therefore properly account for possible structural changes (area/thickness) that are also co-occuring. GABAergic deficits can therefore account for the abnormalities observed in gamma rhythms as well as the disruption of efficient information transfer between brain regions seen in patients.

One potential problem with using neuroimaging exclusively is that the results could always just be probing a non-functionally relevant correlate of the disease. There is therefore a need to demonstrate that there is a behavioural deficit in Sz and that this is also related to changes in our non-invasive biomarkers. Because MRS and MEG markers are so robust for visual cortex measures, we need visual behavioural tasks that probe this at the behavioural level. Certain visual psychophysics tasks such as the orientation discrimination task and the tilt illusion can do this as they are thought to be dependent on neural inhibition and have been previously linked to both MRS and MEG markers.

1.6 Visual processing

The visual system is the largest in the brain and is responsible for processing the visual image. This system is widely studied and thus much is known about its anatomy and structure. Information from the left and right visual fields enters both eyes and is carried along the optic nerve where the information from the

corresponding visual field to that eye crosses over to the contralateral hemisphere through the optic chiasm. Information then travels through the optic tract to the lateral geniculate nucleus (LGN), then to the primary visual cortex in the occipital lobe of the brain for further processing. Thus, information from the right visual field received from both eyes will be processed in the left hemisphere of the visual cortex and vice versa (see Figure 1.6).

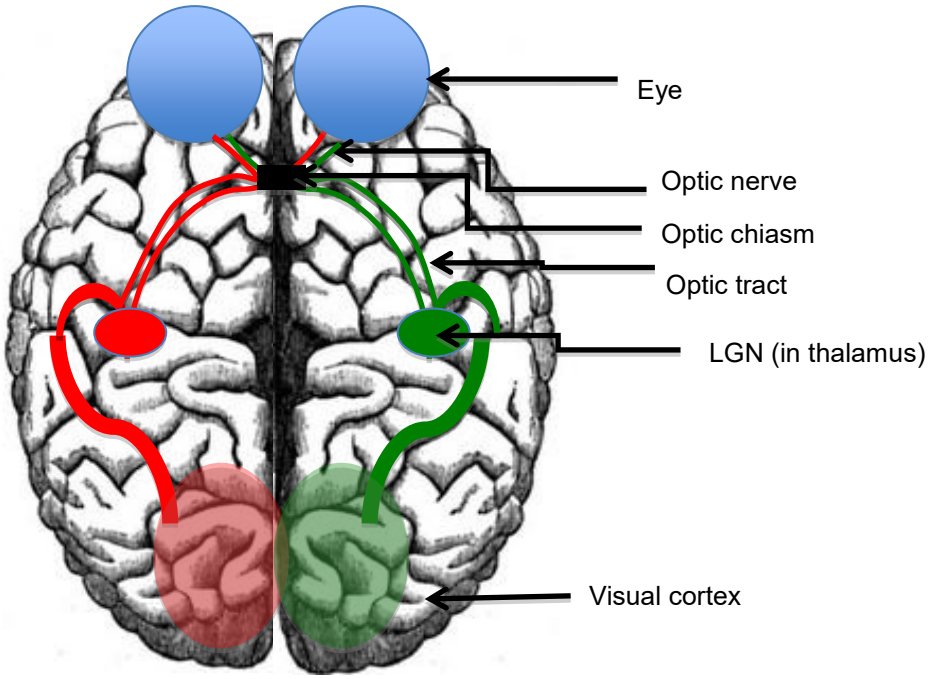


Figure 1.6 Visual system pathway. As shown in the above image the visual cortex receives images from the contralateral eye.

A simple cell in the primary visual cortex is a cell that responds primarily to bars of a preferred orientation (Hubel and Wiesel (1959). These cells were termed simple because they shared the following properties; they have distinct excitatory and inhibitory regions, their excitatory and inhibitory regions balance themselves out in diffuse lighting and it is possible to predict responses of moving stimuli given the map of excitatory and inhibitory regions. Complex cells are also found in V1 and like a simple cell will respond to a preferred orientation, however it has a degree of spatial invariance. This means that its

receptive field cannot be split into excitatory and inhibitory zones. Rather, it will respond to patterns of light in a certain orientation within a large receptive field, regardless of the exact location (Hubel and Wiesel, 1962).

Cells in V1 are also responsive to certain aspects of the visual field; in particular orientation, spatial frequency and colour. Different cells within the visual system such as the ganglion cells within the optic nerve are responsive to different aspects of the visual field; M cells have large centre-surround receptive fields and are sensitive to depth, P cells have smaller centre-surround receptive fields and are sensitive to colour and shape and K cells have very large centre only receptive fields that are sensitive to colour. Information from these cells is sent to V1 for processing and then onto different areas of the extrastriate visual cortex.

1.7 Visual psychophysics

Psychophysical tasks are designed to investigate how changes in stimulus change what we perceive. They are low-level, inexpensive and quick and easy to administer making them useful tasks to administer to challenging groups such as psychiatric patients or participants with reduced cognitive abilities. For the purpose of this thesis I will focus on two psychophysical tasks; the Orientation Discrimination task (OD task) and the Tilt Illusion task (TI task).

1.7.1 Orientation discrimination task

The orientation discrimination (OD) task measures the threshold at which a participant can no longer discriminate the orientation of two sequentially presented grating circles. The starting mean orientation for this task can vary

with cardinal orientations or oblique orientations being used. In healthy control participants, the thresholds when using a cardinal starting orientation are on average 0.5°+/- 0.3° but when using an oblique orientation average thresholds increase by 3.5-5x that of cardinal thresholds to approximately 1.8°+/- 0.5° (Tibber, Guedes & Shepherd, 2006; Edden, Muthukumaraswamy, Freeman & Singh, 2009). This strong performance benefit for cardinal orientations is known as the *oblique effect.* One's ability to perform well on this task is dependent on the orientation selectivity of cells in the visual cortex.

1.7.2 Orientation tuning and neuronal selectivity

The tuning of visual cortical neurons to different orientations is one of the best understood examples of visual selectivity since it was first described by Hubel and Wiesel (1959). Cells within the visual cortex respond maximally to a bar or grating of a given orientation, also known as the cell's preferred orientation (PO). The level of response drops the further the orientation of the stimulus is away from the cell's PO, thereby allowing that cell's tuning curve to be plotted (see Figure 1.7).

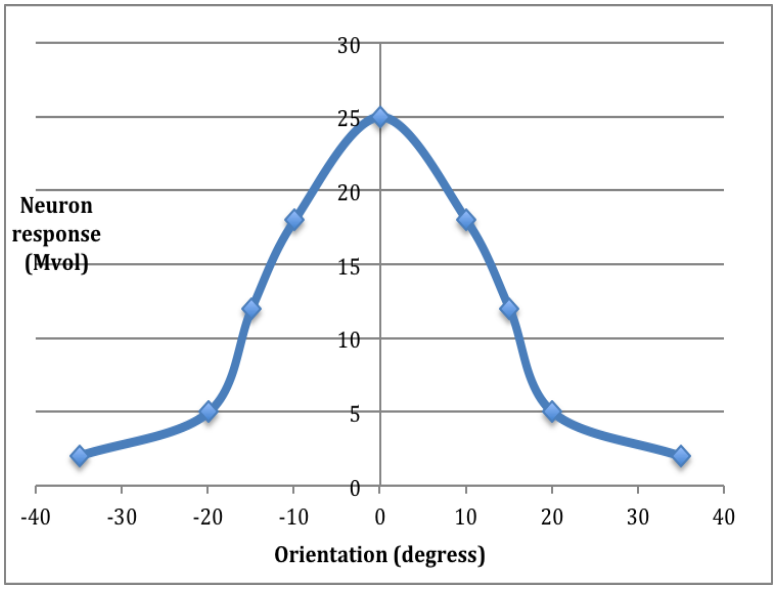


Figure 1.7 Orientation tuning curve of a single cell with a PO of 0° (vertical)

Hubel and Wiesel (1962) developed the early theory of orientation selectivity, which stated that this arose from the convergence of excitatory afferents from the lateral geniculate nucleus (LGN). In this model LGN cells are aligned in a row along the receptive field of the orientation selective neuron and this feedforward connectivity would account for any increase in the response for the cell's PO when compared to other orientations. However, whilst this model was good at predicting a cell's PO it was not good at predicting the tuning curve of orientation selective cells.

Inhibition became of interest for orientation selectivity due to studies showing that the blocking of cortical inhibition, using GABAa antagonists, impacted the tuning of both simple cells (Sillito, Kemp, Milson and Beradi, 1980) and complex cells (Sillito, 1979) to stimulus orientation with the vast majority of simple cells completely losing their orientation selectivity. This, therefore, suggested that

GABAergic inhibition was necessary for the tuning of orientation selective neurons. However, there was the problem of ceiling effects with these studies as they were ultimately suggesting that stimulus-specific inhibition arises from cells with different POs to that of the target cell. Further, Nelson, Toth, Sheth and Sur (1994) showed how excitation is sufficient for orientation selectivity of visual cortical cells. Inhibitory GABA receptors were blocked intracellularly and the tuning curves of 18 neurons were measured. They found all 18 neurons remained selective for their preferred orientations with solely excitatory inputs suggesting inhibition is not necessary for orientation selectivity.

However, inhibition still remained a topic of research with pharmacology research demonstrating that blocking inhibition to orientation selective neurons using GABA antagonists, increased the firing rate of that neuron to non-preferred orientations, thus broadening their tuning curves (Crook, Krisvardy and Eysel, 1998). Troyer, Krakowski, Priebe and Miller (1998) expanded these results to propose a model for orientation selectivity based on cortico-cortical inhibition whereby there are broadly tuned LGN convergent excitatory inputs with more broadly tuned inhibitory inputs which cancel out the wide angle responses and leave a sharper tuning curve.

Support for the cortico-cortical inhibition model comes from several studies demonstrating how administration of bicuculline, a GABAa antagonist, to the visual cortex resulted in diminished orientation selectivity for moderately and strongly orientation selective cells whereas administration of GABA resulted in

improved orientation selectivity within weakly orientation-selective cells along with a slight improvement in moderately orientation-selective cells (Li, Yang, Liang, Xia, Yang and Zhou, 2008; Katzner, Busse and Carandini, 2011).

Since the ability to make orientation-based judgements depends on the width of the tuning curve (Regan and Beverly, 1985; Beaudot and Mullen, 2006), psychophysical tasks that depend on the ability to make orientation judgements, such as the OD task, should demonstrate the strength of cortical inhibition.

Supporting evidence for the role of GABAergic inhibition in orientation judgement using an orientation discrimination task comes from Edden et al., (2009) who showed a negative correlation between in-vivo GABA levels within the visual cortex and OD thresholds thereby demonstrating that increased GABA levels resulted in lower oblique thresholds. They found no relationship between GABA and vertical thresholds, a point that will be taken up in Chapter 3. A recent study (Dickinson, Bruyns-Haylett, Jones and Milne, 2015) investigated the relationship between autistic traits, OD thresholds and peak gamma frequency in a healthy sample. In line with Edden and colleagues findings, they found significant negative correlations between OD thresholds and peak gamma frequency and autistic traits. This lends further support to the idea that inhibition plays a major role in orientation selectivity and performance on the task.

When considering the oblique effect, its neural mechanisms are still under discussion. It has been shown that the oblique effect is not due to the optics of

the eye (Maffei and Campbell, 1970), the retina (Mitchell, Freeman and Westheimer, 1967) or eye movements (Nachmias, 1960), which suggest that the visual cortex or feedforward mechanisms from the LGN are more likely the origins of the oblique effect. In support of this idea, Li, Peterson and Freeman (2003) demonstrated in the cat visual cortex that there are more cells with a preferred orientation of horizontal or vertical compared to oblique angles. In addition, the tuning curves of those neurons preferring oblique angles were much broader. Other studies have shown less cortical area devoted to cells with oblique POs (Coppola, White, Fitzpatrick and Purves, 1998; Wang, Ding and Yunokuchi, 2003) and fMRI studies have demonstrated increased activity in relation to vertical and horizontal angles than to oblique.

Whilst this research all points to a cortical origin of the oblique effect the effect sizes are modest and do not match the size of the oblique effect. A study measuring cortical representation of oblique orientations in visual area 17 in the cat visual cortex showed how this was greatly increased by glutamate excitation in area 21a. Cortical representation was also decreased by GABA inhibition in this area (Liang, Shen and Shou, 2007). This study suggests that the oblique effect may be due to feedback from higher-level visual areas. This could account for Edden et al's (2009) finding being specific to oblique orientations because participants with more GABA within these higher-level visual areas may have increased inhibitory control of these feedback processes. However, work to date has yet to investigate this possibility, as studies addressing this question would need to measure several smaller voxels within the visual cortex, which can have implications for available acquisition time and thus the quality of data.

1.7.3 Orientation discrimination and schizophrenia

Orientation discrimination has also been related to psychiatric disorders and, consistent with findings of a reduction in occipital (visual cortex) GABA levels in patients with schizophrenia, patients have been found to have significantly wider orientation tuning curves compared to healthy controls (Rokem, Yoon, Ooms, Maddock, Mizenberg & Silver, 2011). However, there has been very little research conducted with patients using the OD task discussed previously in this thesis to ascertain whether a deficit occurs and whether an oblique effect is present within this patient group.

1.7.4 Tilt Illusion

The Tilt Illusion (TI) task is a perceptual phenomenon whereby a participants' perception of vertical can be altered by the introduction of a grating surround (Gibson, 1937). This produces either a repulsive or attractive affect depending on the orientation of the surround relative to a central test patch. The surround affecting the perception of a central patch is also referred to as contextual modulation. Repulsion occurs when the surround is oriented between 10° and 25° clockwise or anticlockwise relative to the centre circle with the peak effect occurring at 15°. This is known as the direct effect. Attraction occurs when the surround is oriented between 55° and 90° relative to the centre circle with the peak effect occurring at 70°. This is known as the indirect effect. The results are more pronounced for the direct effect which generally produces a change in perceived tilt of approximately 3° compared to baseline with no surround, whereas the attraction effect usually produces a change of approximately 0.5°.

1.7.5 Contextual modulation

Evidence for a psychophysical theory of contextual modulation first came from Gibson and Radner (1937) who suggested that the direct and indirect effects occur because the inspection of the tilted surrounding stimulus results in the participant adapting to the nearest vertical or horizontal axis. They called this shift in perception 'normalisation'. Thus if a grated stimulus was tilted 15° clockwise from vertical it would be normalised towards vertical so that it appeared less tilted, 10° for example. A subsequent vertical grating would then appear 5° anticlockwise if the 15° difference was still seen. The main problem with this theory is that it cannot account for the fact that the indirect effect is consistently weaker than the direct effect.

To try and explain this difference in the size of effects, lateral inhibition was discussed to account for the direct effect (Blakemore, Carpenter and Georgeson, 1970). Lateral inhibition is the capacity of an excited neuron to inhibit the activity of a neighbouring neuron, thus sharpening the response of that particular neuron. In the relation to the TI, the orientation of the surround causes inhibition from the most excited orientation neurons, which in turn produces a short-lived reduction in the output of neurons that prefer neighbouring orientations. Thus when the surround is present the neurons giving the greatest output may not be those whose preferred orientation corresponds to the centre circle, thus producing the shift in orientation perception. The idea was that the direct effect was the sum of both this and normalisation, accounting for the stronger effect, and the indirect effect was the result of normalisation alone.

The idea of lateral inhibition was extended to include disinhibition as an explanation for the indirect effect, a process termed gain control. Specifically, inhibition between neurons tuned to specific orientations at the same location, as well as those tuned to specific orientations but at different locations, can lead to disinhibition of neurons remote in both position and orientation (Clifford, 2014). This in turn leads to the responsiveness of the orientation selective neurons being normalized by the responses of similar neurons (Goddard, Clifford and Solomon, 2008). It is an appealing idea as it refers to a process that allows, in this instance, the visual system to adapt its responses to stimuli to take into account spatial and temporal context (Butler, Silverstein and Dakin, 2008). In the gain control model of the TI, the magnitude of disinhibition never exceeds that of inhibition. Therefore, the magnitude of the indirect effect (disinhibitory) will never exceed that of the direct effect (inhibitory) thus accounting for the difference in magnitude of effects.

There is now a growing body of evidence that the direct effect can occur with no conscious awareness, but the indirect effect cannot. Tomassini and Solomon (2014) prevented awareness of the stimulus in his participants by using adaption-induced blindness and demonstrated how a strong direct effect, using a surround of 15° relative to the centre, remained whereas the indirect effect, using a surround of 70°, disappeared. This lends further support to the idea of different mechanisms for each effect.

1.7.6 GABA and Tilt illusion

Context modulation has been linked with GABA by Yoon et al., (2010), who reported patients with schizophrenia had lower levels of GABA in the visual cortex and also showed weakened contrast gain control, as evidenced by reduced orientation-specific surround suppression. These results were irrespective of anti-psychotic medication use (calculated using chlorpromazine equivalents).

The TI specifically has also been linked to GABA as work using Lorazepam, a GABA potentiating benzodiazepine, showed a dose related increase in the magnitude of the direct effect (Gelbtuch, Calvert, Harris and Phillipson, 1986). In other words the higher the participants' GABA levels were, the more impact the surround had and the bigger the direct effects. However, this only occurred for the TI, as Lorazepam dose had no effect on the tilt after effect, suggesting that the link with GABA is specific to particular visual phenomena. This result fits quite neatly with the finding of a reduction in GABA in patients with schizophrenia and the psychophysics literature, which has shown for different surround suppression tasks including contrast, motion, orientation etc, that the introduction of the surround has little to no effect on patients.

1.7.7 Contextual modulation and schizophrenia

The level of gain control is proposed to be weaker in patients with schizophrenia than in controls (Butler et al, 2008; Phillips and Silverstein, 2013) leading to a reduction in contextual modulation as demonstrated by Dakin, Carlin and Helmsley (2005); Serrano-Pedraza, Romero-Ferreiro, Read, Diéguez-Risco, Bagney and Caballero-González (2014) who found reduced

contextual modulation in a patient group when measuring perceived contrast and by Yoon, Rokem, Silver, Minzenberg, Ursu, Ragland and Carter (2009) when measuring orientation selectivity. Results from Yang, Tadin, Glasser, Hong, Blake and Park (2013) show normal contextual modulation of luminance but impaired modulation of contrast which provides further evidence of dysfunctional inhibitory, possibly GABAergic, circuitry in the early visual cortex.

Imaging studies have supported the idea of reduced levels of contextual modulation in schizophrenia with recent fMRI studies providing strong neurophysiological evidence for this theory having shown abnormally weak orientation specific surround suppression in the visual cortex of patients with schizophrenia (Seymour, Stein, Sanders, Guggenmos, Theophil and Sterzer, 2013). From the results referenced above it would be expected that patients would have weaker direct effects when using the TI task. However, when investigating the direct effect in patients the results are mixed. Both Yang et al (2013) and Tibber, Anderson, Bobib, Antonova, Seabright, Wright et al (2013) found no difference between patients and controls in the magnitude of the direct effect. In addition, Yang et al (2013) found that larger direct effects within the patient group were associated with higher symptom severity whereas Tibber and colleagues (2013) found the opposite direction of results.

These results are contrary to what intuitively would be expected given the results of Butler et al, 2008; Philips and Silverstein, 2013 and Dakin et al., (2005), and also Gelbtuch et al (1986) who positively correlated GABA with the size of the direct effect in healthy controls. Given the previous findings of a reduction in GABA levels in patients with schizophrenia it would be expected

that this group would demonstrate weaker contextual modulation compared to healthy controls thereby resulting in weaker direct effects. There is limited specific research into the TI task and schizophrenia. More research is needed using this task within this patient group to investigate how contextual modulation relates to this disorder and how this could be mediated by neurophysiological factors.

1.8 Objectives

The measurement of neural activity, neurotransmitter concentration and behaviour is essential for the understanding of how this behaviour arises in both healthy and disease populations. This thesis will investigate group differences in gamma band activity using MEG methods to achieve a time-resolved measure of oscillatory dynamics, in-vivo GABA levels using MRS methods and psychophysical methods, specifically the orientation discrimination task and the tilt illusion, to derive a behavioural measure known to relate to both the imaging outcomes. The first experimental chapter investigates how stimulus duration affects orientation discrimination thresholds in healthy controls. The second investigates the reliability of the tilt illusion task and how the results relate to visually induced gamma frequency and amplitude. The third examines whether there is a between group difference in GABA levels in an occipital (visual) voxel and a sensory motor voxel for comparison. The fourth investigates between group differences in gamma measures using two visual tasks. The final experimental chapter aims to bring the experimental measures together in order to look at the relationship between groups for the imaging and psychophysics measures. The hope is that if a relationship does exist between the imaging and psychophysics, then instead of patients undergoing lengthy and expensive

imaging scans these behavioural measures could be developed as potential biomarkers and used in a clinical setting as a proxy measure for levels of cortical inhibition.

Chapter 2 - Methods

2.1 Psychophysics

2.1.1 Orientation discrimination

The Orientation discrimination task is designed to determine a participant's threshold for discriminating the orientations of two sequentially presented circularly-windowed gratings of different orientations (Edden et al., 2009). For example stimulus see Figure 2.1. Participants are instructed to respond which of two sequentially presented grated circles is orientated more to the right in a two-alternative forced choice design. Thresholds are calculated using two adaptive staircases running simultaneously with both converging on ~71% correct performance. A 2up-1down staircase system is used whereby the participant has to answer correctly twice in a row for the task to get harder (orientation difference between the two circles gets smaller) but only have to answer incorrectly once for the task to get easier (orientation difference between the two circles gets bigger). The task switches randomly between the two staircases so that participants cannot monitor or predict upcoming trials. The task will continue until there have been 12 reversals in each staircase with thresholds calculated only from the last 10. These are then averaged to get an overall orientation discrimination threshold.

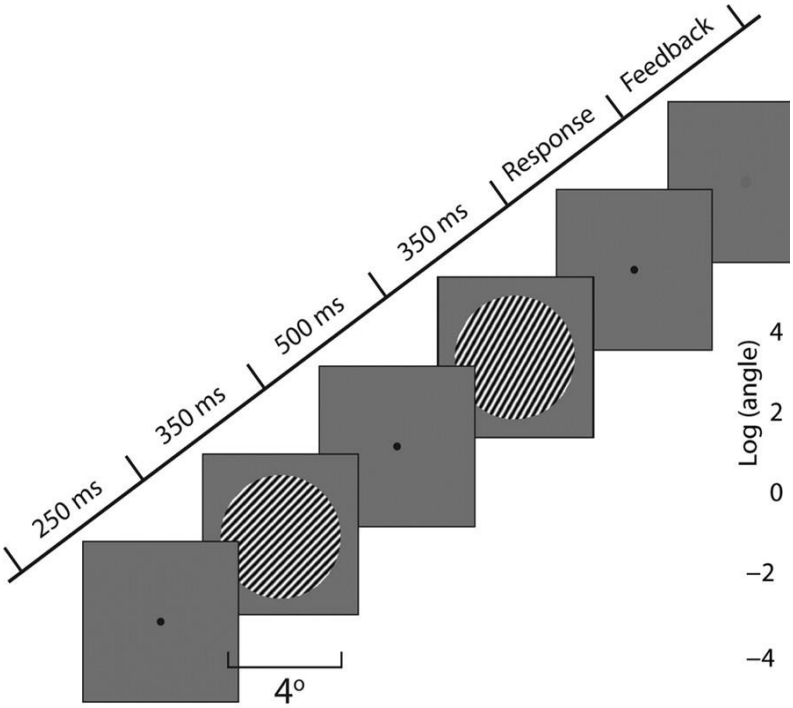


Figure 2.1 – Orientation discrimination experiment (adapted from Edden et al, 2009)

2.1.2 Tilt illusion

The tilt illusion is designed to measure to what extent the perceived orientation of a test grating is altered by the introduction of a surrounding grating of different orientations (Gibson, 1937). For an example of the stimulus with surround see Figure 2.2. Two conditions are performed; one with an oblique surround and one with no surround, which is considered as a baseline measure, with each block split into two parts. The first part uses a Method of Adjustment (MA) design to calculate what the participant perceives as vertical over four trials. Participants are presented with a flashing circularly-windowed grating stimulus of different orientations and are instructed to rotate the stimulus, using mouse button presses, until they perceive the lines as being vertical. The mean orientations from each of these trials are then averaged to get a mean perceived vertical for that participant.

The second part of the task is designed to assess how well the participant can judge the orientation of a quickly presented circularly-windowed grating stimulus. The stimulus used is the same as in the first part of the task but is only presented once. A Method of Constant Stimuli (MOCS) procedure is used for the second part of the task. The mean perceived vertical output is used as a mid point for seven orientations, with a fixed step size, from which participants must judge whether they are left or right of vertical. These orientations would be presented randomly for a set number of trials each.

A psychometric function is calculated with the Point of Subjective Equality (PSE) being the output of interest. In this instance the PSE refers to the point at which the participant can no longer discriminate between left and right and perceives the test grating as vertical. It is the shift or difference in the PSE between the no surround/baseline condition and the surround condition that is recorded and referred to as the direct effect (Gibson, 1937)

Figure 2.2 – Example Tilt Illusion stimulus with surround

2.2 Imaging Methods

2.2.1 Magnetoencephalography

Magnetoencephalography (MEG) is a non-invasive functional brain imaging technique used to measure electromagnetic activity generated by synchronised neuronal firing within the brain (Singh, 2006). It has both high temporal and spatial resolution of neuronal activity, something MR imaging does not have.

2.2.1.1 MEG signal generation

The externally measured MEG signal is generated by post-synaptic potentials (PSPs) within pyramidal cells. Electric currents are generated through these action potentials changing the permeability of the post-synaptic membrane to potassium (K+) and calcium (Ca+) ions. This electric current creates a magnetic field perpendicular to the current, which when measured outside the head constitutes the MEG signal. The magnetic field generated by a single neuron is extremely weak, a factor of 1 million to 1 billion smaller than environmental magnetic noise (Vbra and Robinson, 2001), and would not be detectable on its own thus the MEG signal is the synchronised firing of up to tens of thousands of neurons. Environmental noise cancellation is, therefore, a major problem when using MEG. Magnetically shielded rooms (MSR) are used to stop as much environmental interference as possible with superconducting devices needed inside the room to filter the noise further and measure the signal with adequate sensitivity.

Superconducting QUantum Interference Devices (SQUIDs) were developed to be able to detect such small magnetic fields. Modern MEG systems consist of hundreds of SQUIDs arrayed around the head – the system used in this thesis

has 275, As SQUIDS are superconducting and must be constantly super cooled and stored in liquid helium, the signals received from these sensors are transmitted to room temperature and amplified. Each SQUID sensor although very sensitive, consists of a small loop of material that only collects flux from a small area. For this reason, SQUIDs used in MEG are inductively coupled to a larger set of coils. These are also superconducting and consist of pickup coils containing a single loop of wire. This wire within the pickup coil acts as a magnetometer and is sensitive to magnetic fields perpendicular to its area but also to environmental magnetic noise. Two magnetometers with wires of opposing orientations can be joined together and connected to the same SQUID sensor to create a first-order gradiometer, which is sensitive to the spatial gradient of the field. As magnetic fields rapidly decay the further from the source they become, gradiometers are less sensitive to signals from distant sources compared to the signals generated from activity within a closer source; in this example the brain. Second and third-order gradiometers, using increasingly complex combinations of coils, produce even better signal to noise but are bulky, difficult to manufacture and reduce the signal from deeper brain sources. For these reasons most MEG systems use first-order gradiometers within the scanner itself and higher order gradiometers synthesised in software for further noise cancellation purposes (Vrba and Robinson, 2001).

2.2.1.2 MEG analysis

Once the SQUID sensors collect data, the signals need to be converted from sensor space (the position of the sensors) to source space (the brain). There are two main problems with this process; the forward problem and the inverse problem. The forward problem refers to reliably identifying the magnetic field

generated from a particular source of known position, magnitude and orientation – this is known as the lead field for this location and, if known, allows the simulation of what magnetic field this current source would generate in every SQUID sensor.

The inverse problem is more problematic and refers to being able to reliably determine the location of electrical activity within the brain from the magnetic fields measured outside the head. This has no unique solution, as there are an infinite number of solutions that could generate the recorded electrical activity. A solution can only be produced by the addition of extra *a prior*i information regarding the spatial and temporal nature of the source distribution within the head. Several different approaches to solving the EEG/MEG inverse problem have been developed and are currently in common use. The solutions yielded by each approach depend both on the data and their underlying assumptions about the source.

Beamforming is an analysis tool that creates a 'virtual sensor' over a particular area of interest. The timeseries of the current at this virtual sensor is estimated as a weighted sum of the timeseries of each MEG sensor. Synthetic aperture magnetometry (SAM; Vrba and Robinson, 2001) is the beamformer technique that is used throughout this thesis. SAM makes two assumptions; First, that data are generated by discrete dipolar sources and second, that the time series of these sources are not temporally correlated. Thus, SAM analysis is not recommended for experiments where discrete sources are known to be highly temporally correlated over significant periods of time. Data is split into baseline and stimulation blocks with t-tests calculated to determine if there is any

difference in activity between the two for each virtual sensor.

An important consideration of MEG data analysis is whether the experiment is measuring evoked or induced responses. Both experimental designs tend to repeat a stimulus over many trials and average the signal over all the trials. Evoked responses are phase-locked to the stimulus onset. When all the trials are analysed, the peaks and troughs (i.e. the phase of the response) line up during analysis. This type of evoked analysis is referred to as event-related field (ERF) analysis. Alternatively, some responses are time-locked but not phase-locked to the stimulus. Because the phase of the response is essentially random on each trial, simple averaging of response over trials will result in little or no average response. For this type of data, frequency or time-frequency estimation is needed. This is done by analysing the amount of power within a certain bandwidth before averaging the signal. This type of response is referred to as *induced* with increases in power termed event-related synchronisation (ERS) and decreased power termed event-related desynchronisation (ERD) (Singh, 2006). This thesis will focus on induced responses. Beamformers are particularly well suited to localalise changes in induced oscillatory power, which is why this method is used throughout the thesis.

In order to produce time-frequency plots of induced data from the virtual sensor of interest, it has to be subjected to a spectral estimation based on the Hilbert transform, which yields the time-varying envelope of band-pass filtered data (Figure 2.3). By band-pass filtering the data into many different ranges, each 8Hz wide, centred on increasing frequencies, these HIlbert envelopes can be

‘stacked’ to generate a time/frequency/amplitude spectrogram of the data. These can be baselined to show percentage changes from a defined baseline period (Figure 2.4)

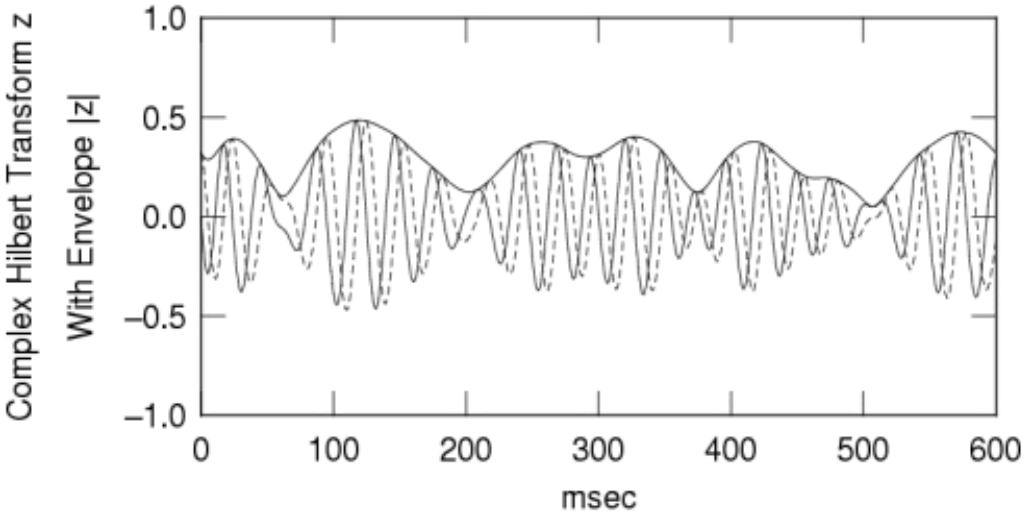


Figure 2.3 Procedure for fitting the positive amplitude peaks to the Hilbert transform envelope.

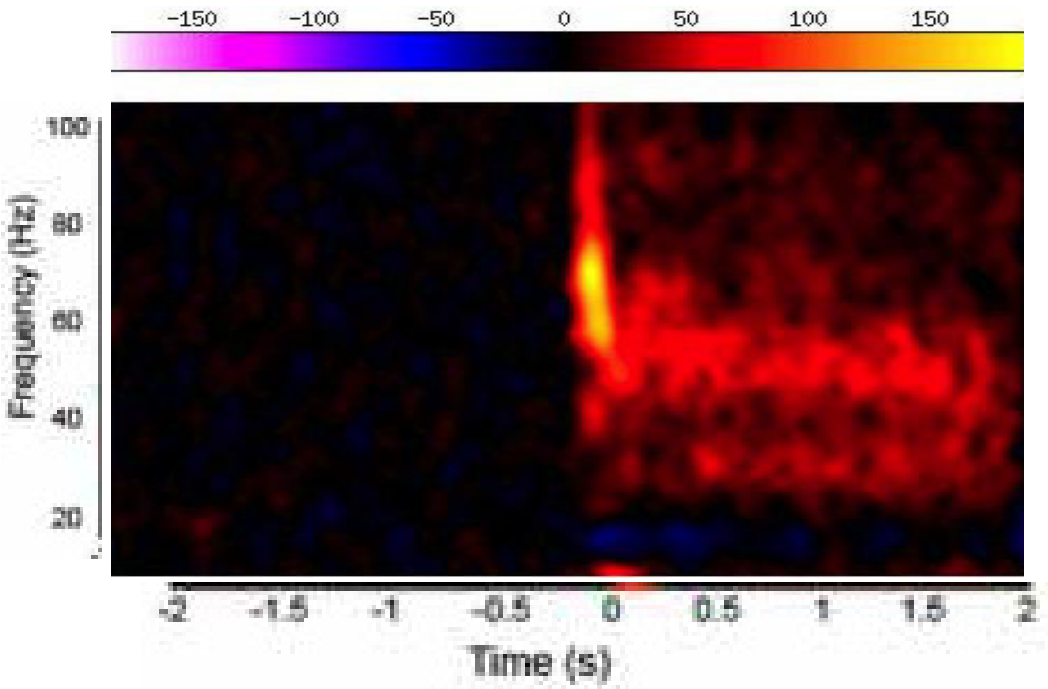


Figure 2.4 Example time/frequency/amplitude spectrogram generated by multiple Hilbert amplitude envelopes. From this spectrogram we can define time/frequency region-of-interests (ROI) (such as the visual gamma response) and calculate summary measures, such as the peak amplitude and frequency of the response, within each ROI.

2.2.2 MRI signal

MRI takes advantage of different molecules behaving differently when exposed to a strong magnetic field. Hydrogen is most widely used because there is a high quantity of it in the body (~70% of the body if made up of water- H_2O) and only nuclei with an odd number of protons produces a magnetic field. This is referred to as ^1H MR imaging.

Hydrogen protons spin on their own axes pointing in different directions but when exposed to a strong magnetic field they align with this magnetic field in two ways, parallel and anti-parallel. The distributions of parallel and anti-parallel atoms are not the same, as atoms prefer to be in the parallel state pointing in the same direction as the magnetic field. Therefore, there are slightly more atoms aligned parallel to the magnetic field, the sum of which is referred to as the net magnetisation. When exposed to this strong magnetic field the protons precess (wobble) at a define frequency due to the magnetic moment of the proton at the Larmor frequency. This frequency is given by the gyro/magnetic ratio (MHz/T) multiplied by the strength of the magnetic field (B0) measured in Tesla (T). This equation is important as it calculates the operating radio frequency of the MRI system (Blink, 2004).

When the atoms are presented with a RF pulse at the resonant frequency described by the above Larmor equation, the net magnetisation can be rotated into a plane perpendicular to the magnetic field (see Figure 2.5). This process is called excitation and can also be explained as shifting the protons into a higher energy state through them absorbing the energy from the RF pulse. Protons will decay back to the lower state, aligning with the magnetic field and in doing so

release the absorbed energy in the form of RF energy at the same frequency – this is known as relaxation.

Once the RF pulse stops the net-magnetisation will now rotate back along the direction of the magnetic field. As the signal measured by the RF coil is generated by the transverse magnetisation, this will appear to decay with a time constant known as T1. T1 relaxation is defined as the time it takes for the longitudinal magnetisation to recover 63% of the original magnetisation. This will depend on the molecule as well as the tissue type as hydrogen atoms are bound very tightly in fat tissue but much looser in water. Tightly bound protons will release their energy a lot quicker than those that are bound loosely. As well as the T1 relaxation time, another fundamental mechanism of MR signal relaxation is governed by the T2 decay constant. T2 relaxation occurs because although all the nuclear (proton) spins are initially in phase after RF excitation, generating a bulk coherent signal, as time progresses random dephasing of the spins occurs leading to a weaker, non-coherent, signal. This dephasing is also reflective of the local tissue characteristics.

The emission of these radio frequencies are received and measured by a receiver coil placed over the area being scanned. The contrast between different tissues can be generated by MR sequences that are designed to be sensitive to different aspects of the T1 and T2 decay processes (Hashemi, Bradley and Lisanti, 2010).

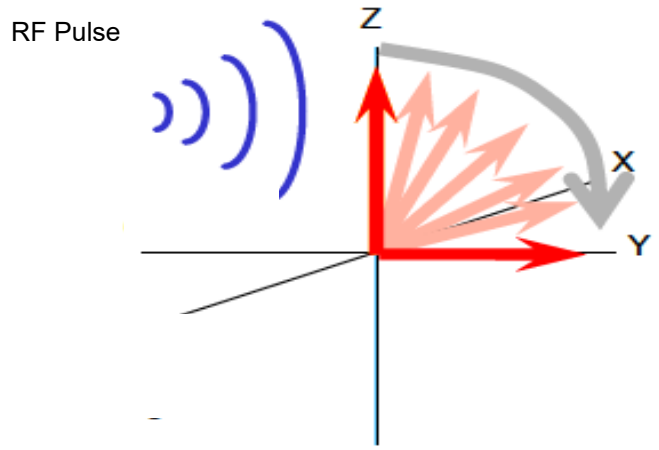


Figure 2.5 Excitation from RF pulse causing rotation of net-magnetisation

2.2.3 Magnetic Resonance Spectroscopy

Magnetic Resonance Spectroscopy (MRS) uses the same principals as MRI, but focuses on producing information regarding concentrations of different molecules within the voxel of interest. ^{1}H MRS detects signals that arise from hydrogen spins within tissue metabolites after being subjected to a strong magnetic field. These signals have chemically specific resonance frequencies as the chemical environment of the hydrogen spins cause small modulations of the main magnetic field (Puts and Edden, 2012). For example, if the electrons within the molecule are relatively close to the hydrogen atom then this has a shielding effect resulting in the proton being subjected to a weaker magnetic field. This is known as the Chemical shift. As each molecule will have its own unique resonant frequency, which is consistent over time, MRS can be used to focus on certain peaks (metabolites) of interest.

Chemical shift is reported on the Chemical shift axis, or MRS spectra (see Figure 2.6). The x axis is plotted from high to low frequency and reports the chemical resonance spectrum in parts per million (ppm), quantified as the difference in frequency between the molecule of interest and a reference (usually water or creatine as these are generally stable over time and easy to distinguish from other molecules in the MRS spectra), divided by the operating frequency of the scanner, with the y axis reporting signal intensity corresponding to proportional metabolite concentration. Molecules, or metabolites, with more electrons and more shielding will therefore be on the lower end of the spectrum.

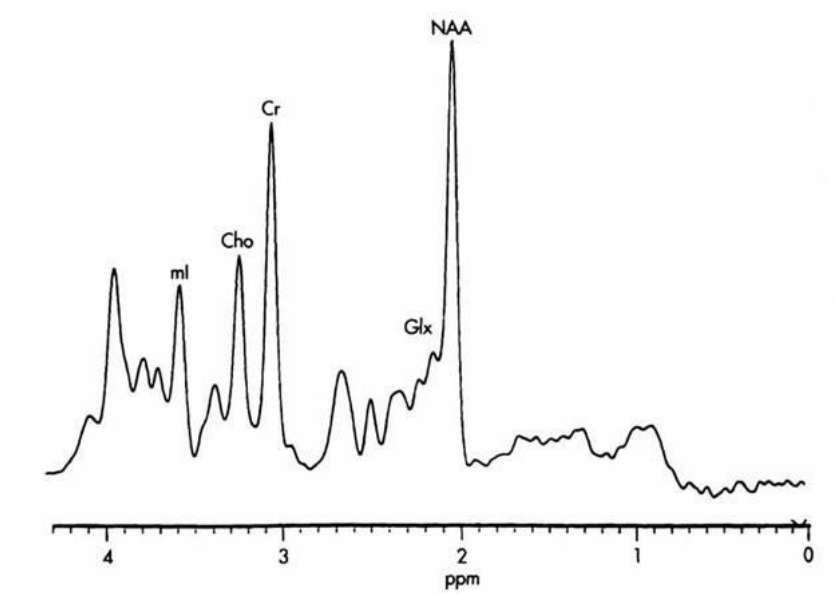


Figure 2.6 Example MRS spectra/Chemical shift axis

Quantification of the reference molecule is acquired through internal referencing, whereby both the molecule of interest and the reference are acquired from a voxel within the participant during the same scanning session. This minimises possible confounders such as participant movement, voxel position and possible software or hardware abnormalities between scans.

In order to get good quality spectra the signal to noise ratio needs to be high to get good height on the signal peaks. This can be increased by longer acquisition times; over 5 minutes preferably, larger voxel size to obtain more signal; typically 8cm³, or the positioning of the voxel to try to exclude as much bone, cerebrospinal fluid (CSF) or water as possible, as these can affect the quality of the signal (Provencher, 2001).

2.2.4 MRS GABA analysis

Isolating one particular metabolite is not easy as some can overlap on the same point in the spectra (e.g. GABA and Creatine both peak at 3ppm) or the same metabolite can spread across the spectra at lower intensities. The latter is known as multiplets or coupling and are associated with signals from a single hydrogen environment that are split into a number of sub-peaks, for example GABA also appears at 1.9ppm and 2.3ppm on the MRS spectra.

To filter out specific metabolites, spectral editing analysis tools can take advantage of the fact that coupling occurs and can be used to separate signals from the rest of the spectra. The GABA peaks at 1.9ppm and 3ppm are coupled whereas other signals at this point in the spectra, such as creatine, are not meaning it is possible to separate the signals. A frequency-selective pulse targeted at 1.9ppm would also indirectly affect the GABA 3ppm signal without affecting the other metabolites due to this coupling. MRS experiments will, therefore, conduct two experiments; one with and one without the frequency-selective pulse (also known as the editing pulse) and take the difference between the two. This will give an edited spectrum that contains only those signals that are affected by the editing pulse; signals that occur at 1.9ppm and

those arising due to coupling (Puts and Edden, 2012). This technique is referred to as J-difference editing and is used in the mostly widely used method for analysing GABA data; MEGA-PRESS (Mescher, Merkel, Kirsch, Garwood and Gruetter, 1998), which is used in this thesis. Although this method does have its issues, as the quality of the spectra can be greatly affected by participant movement, for example, it is the best measure of GABA currently possible and has been validated by correlation with chromatographic measurements of GABA (Bielicki, Chassain, Renou et al (2004).

2.2.5 Recruitment of participants with schizophrenia

The patient group for this thesis was recruited from a previous Cognition in Psychosis study research conducted at Cardiff University (Schizophrenia Working Group of the Psychiatric Genomics Consortium, (2014). As part of participating in this project, researchers would administer the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview. This went through core symptoms associated with psychosis, depression, mania and anxiety. The interviewer would rate each symptom with a provisional diagnosis given after completion and review of the interview as a whole. The diagnosis would then be verified by clinical consensus rating in line with the DSM-V (American Psychiatric Association, 2013). For this thesis only those with a DSM-IV diagnosis of schizophrenia were recruited. Of those patients that had given consent to be re-contacted about future projects, those that had this verified diagnosis were contacted by a letter of invitation with a reply slip included. Once I had received the reply slips indicating they would like more information, I would contact the participant directly by phone.

Chapter 3 - Individual variation in orientation discrimination thresholds as a function of stimulus duration

3.1 Rationale

Performance in psychophysical tasks such as orientation discrimination have been linked to GABA inhibition, in vivo GABA levels and gamma frequency in healthy controls but there is a potential problem with the task itself. In this study we investigated whether the results in previous research were confounded by possible ceiling effects.

3.2 Background

The orientation discrimination task asks participants to discriminate the orientation of two sequentially presented circular grating patches to obtain a threshold for discrimination for that participant. It is easy to administer and short in duration making it appropriate for both healthy and patient populations.

The use of this task usually consists of cardinal conditions, where the mean angular difference between two test patches will equal either 0°, 90° or 270°. Oblique conditions are also used which usually use a mean angular difference of 45° or 315°. The human, and animal to some extent, visual system is much more finely tuned to cardinal orientations, in particular in the horizontal plane, than to oblique orientations (Li et al., 2003). Thus, discrimination thresholds calculated in healthy controls using cardinal conditions are generally between 0.5-1.5°, but when using oblique orientations participants' thresholds are 3-5 times higher generally being between ~1.6-3.3° (Appelle, 1972; Vogels and

Orben, 1985; Tibber et al., 2006; Edden et al., 2009). In a select few participants, the oblique condition is simply too hard to complete to a satisfactory standard with results having to be discarded (Edden et al, 2009). The reason for such a strong 'oblique effect' is still relatively unknown, with animal work suggesting it's simply due to more neurons within the visual system being tuned to cardinal orientations than oblique (De Valois, Yund & Hepler, 1982; Celebrini, Thorpe, Trotter & Imbert, 1993) or more cortical area is dedicated to cardinal orientations compared to oblique (Coppolo et al., 1998; Xu, Collins, Khaytin, Kaas, & Casagrande, 2006).

The oblique effect has been linked to GABAergic processes, with one theory suggesting GABA inhibition influences the oblique effect by acting on higher-level visual areas. After administration of GABA to area 21a of the cat visual cortex, the areal representation of cardinal orientations decreases compared to oblique in the visual cortex, abolishing the oblique effect (Liang et al., 2007; Shen, Liang & Shou, 2008). This suggests a feedback mechanism from higher-level areas within the visual cortex contributes to the oblique effect. Thus, if a participant had increased levels of GABA in the visual cortex they would have increased levels of inhibition, reducing the oblique effect in these individuals.

This link between the oblique effect and GABA could account for results obtained by Edden et al (2009), who showed resting GABA negatively correlated with oblique discrimination thresholds in healthy controls. However, this relationship was not significant when looking at vertical orientation discrimination thresholds. This result suggests increased levels of GABAergic

inhibition could lead to increased control over inhibitory mechanisms leading to a reduction in the oblique effect.

The lack of relationship with vertical thresholds could be for a number of reasons including measurement error or lack of between-subject variation leading to very similar results. However, it could also indicate a problem with the task itself. Ceiling effects could be present whereby the vertical condition administered is too easy and therefore wouldn't be able to tap into the variation within the sample. As far as I am aware, there has been no research in how to increase the difficulty of this task and investigate these possible ceiling effects.

3.3 Aims and hypotheses

The purpose of this research was to investigate why this lack of relationship between GABA and vertical thresholds occurred. Consequently, this experiment also refined this task within a healthy control sample. One further problem with this task was the oblique condition was simply too hard for some participants to complete. Therefore we also investigated whether there was an equivalent vertical condition that produces comparable thresholds to the oblique condition without the associated loss of data.

To increase the difficulty of the task for vertical stimuli, we progressively reduced the length of time they were presented and compared performance to an oblique condition presented for the same duration as Edden et al (2009). It was hypothesised this decrease in stimulus duration would increase the difficulty of the task and therefore increase the discrimination thresholds. The relationships between thresholds of each condition were investigated to show if

ceiling effects were present at the slower durations. Finally, vertical conditions will be compared to the oblique condition to see if any of the thresholds are comparable.

3.4 Methods

3.4.1 Participants

Participants used were majority undergraduate students recruited through the School of Psychology's Experimental Management System (EMS) for course credit (n=37) with the remaining participants recruited opportunistically from CUBRIC, also within the School of Psychology (n=9). The sample as a whole was males n=6 and females n=40 (mean age = 20.91 years of age).

3.4.2 Inclusion and exclusion criteria

The inclusion criterion was having normal to corrected vision and aged 18-65. The exclusion criterion was having a diagnosis of photosensitive epilepsy.

3.4.3 Stimuli

Orientation discrimination thresholds were measured using a two-alternative forced choice procedure as shown in Figure 3.1. Stimuli were programmed using DELPHI and consisted of two sequentially-presented circular grating patches (width 4°, contrast 80%, 3 cycles per degree, refresh rate 80Hz, mean luminance 44.5 cd/m^2). The task used vertical conditions where the mean orientation between the two patches was kept at 0°. There was also an oblique condition where the mean orientation between the two patches was kept at 45°.

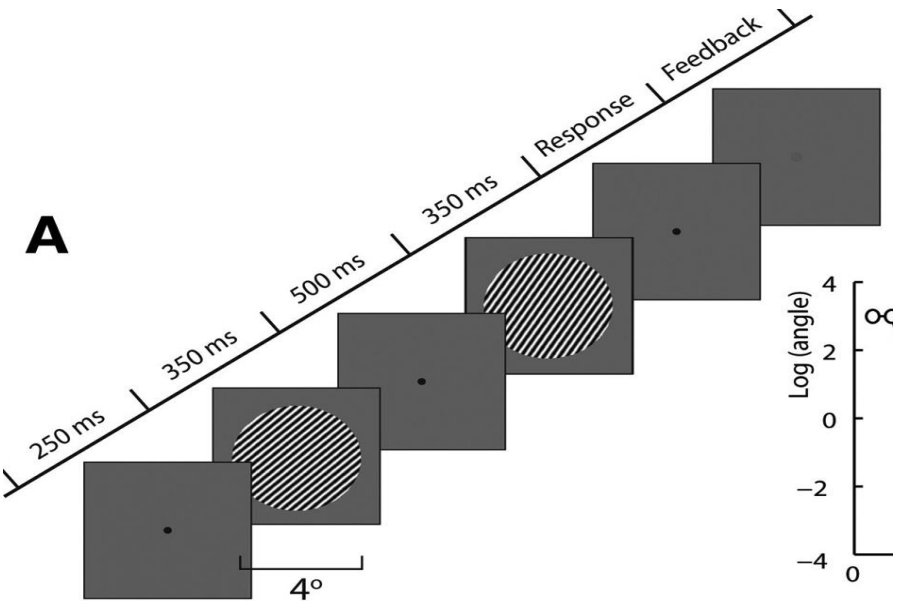


Figure 3.1 – Task outline obtained from Edden et al (2009)

One trial consisted of a fixation dot presented for 250ms, followed by a circular grating test patch, a second fixation spot for 500ms, a second test patch and finally a response fixation spot. The final fixation spot had no time limit and the task only progressed once the participant had responded. The task gave feedback to the participant by beeping once and flashing a green dot should the participant respond correctly to that trial.

The difference in orientation between the two test patches in each trial was determined using two randomly-interleaved 1-up 2-down adaptive staircases. This is described in detail in Chapter 2 section 2.1.1. Thresholds were calculated from the average of the last 10 reversals of both staircases in log steps, which were then converted to linear units in degrees (See Figure 3.2).

Each staircase was locked to 5-log units maximum angular difference (i.e. 32 degs) to avoid large orientations that might be called at the beginning of each session wrapping around.

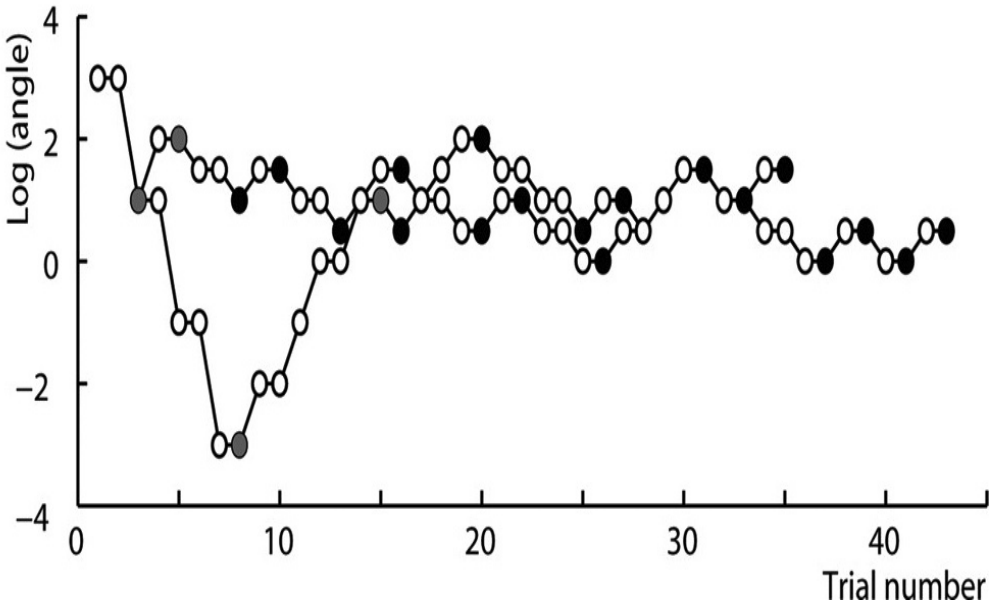


Figure 3.2 – Staircase procedure obtained from Edden et al (2009)

Participants were seated 54.3cm from the stimulus presentation screen and asked to put their chins on a chin rest to stabilize their head and standardise the distance from the screen between participants as best as possible. The room was kept in complete darkness to try and block as many external orientation cues as possible, which may have influenced participants' performance.

3.4.4 Procedure

Informed consent was obtained from participants prior to being seated in the testing room. Participants were asked to respond which of two sequentially presented grating circle patches was oriented more to the right and to respond with a left mouse button press to indicate the first interval and a right mouse

button press to indicate the second interval. Each participant was required to complete a practice condition for both the vertical and oblique conditions before continuing to the full test block. There were 6 conditions in total each with different mean orientations or stimulus presentation times; 10ms, 36ms, 60ms, 100ms and 250ms vertical conditions and an oblique condition of 250ms. Each condition was completed with a short break in between with a verbal and written debrief given on completion of the task. The task took approximately 30 minutes to complete.

3.5 Results

Thresholds were excluded from further analysis if they were 2 standard deviations above or below the mean for that condition. Table 3.1 shows the remaining number of thresholds for each condition.

Condition	% remaining
Vertical 10ms	85%
Vertical 36ms	83%
Vertical 60ms	87%
Vertical 100ms	80%
Vertical 250ms	80%
Oblique 250ms	70%

Table 3.1 – Remaining number of thresholds after discarding those +/- 2 standard deviations from the mean for each condition administered

Figure 3.3 shows the mean thresholds for each condition. A within-subject repeated measures ANOVA showed a significant main effect of time on

orientation discrimination thresholds within the vertical conditions (F(4),26 = 9.613, P=<.000). The mean threshold difference between the 250ms oblique condition and the 250ms vertical condition was also highly significant (t(28) = -8.219, p=0.001 The 250ms oblique condition also produced much higher thresholds than the 10ms vertical condition (t(27) = -5.708, p=<.001).

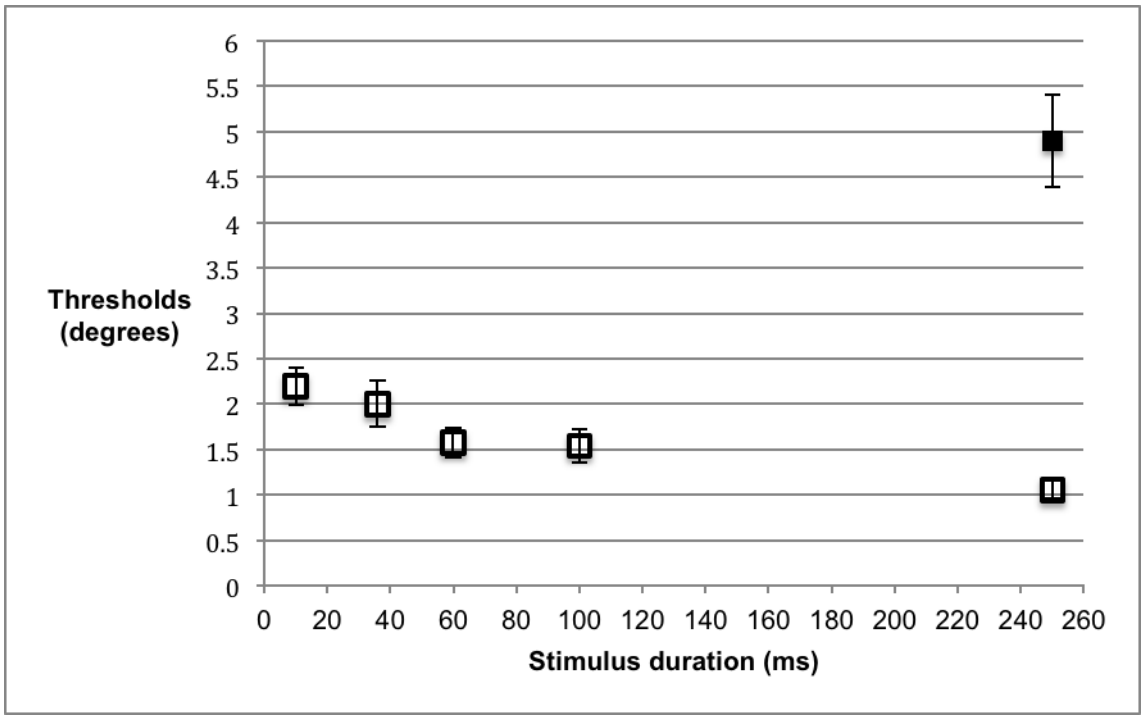


Figure 3.3– Mean thresholds across stimulus durations. Error bars show +/- 1 standard error of the mean. Solid data point represents the oblique condition.

Figure 3.4 shows the correlations between each of the vertical conditions and the oblique condition. Each of the vertical conditions correlated across individuals (see Table 3.2). The 10ms and 250ms conditions in particular were very highly correlated (r=0.825, p=0.000). All vertical conditions moderately correlated with the oblique condition with 100ms the only condition reaching significance.

	10ms vertical	36ms vertical	60ms vertical	100ms vertical	250ms vertical	250ms oblique
10ms vertical		0.771*	0.638*	0.667*	0.825*	0.446
36ms vertical			0.679*	0.405	0.582*	0.402
60ms vertical				0.597*	0.632*	0.498
100ms vertical					0.567*	0.721*
250ms vertical						0.560
250ms oblique						

Table 3.2 – Pearson correlations across all stimulus durations and orientations. *=p<0.001 (p-value corrected for multiple comparisons)

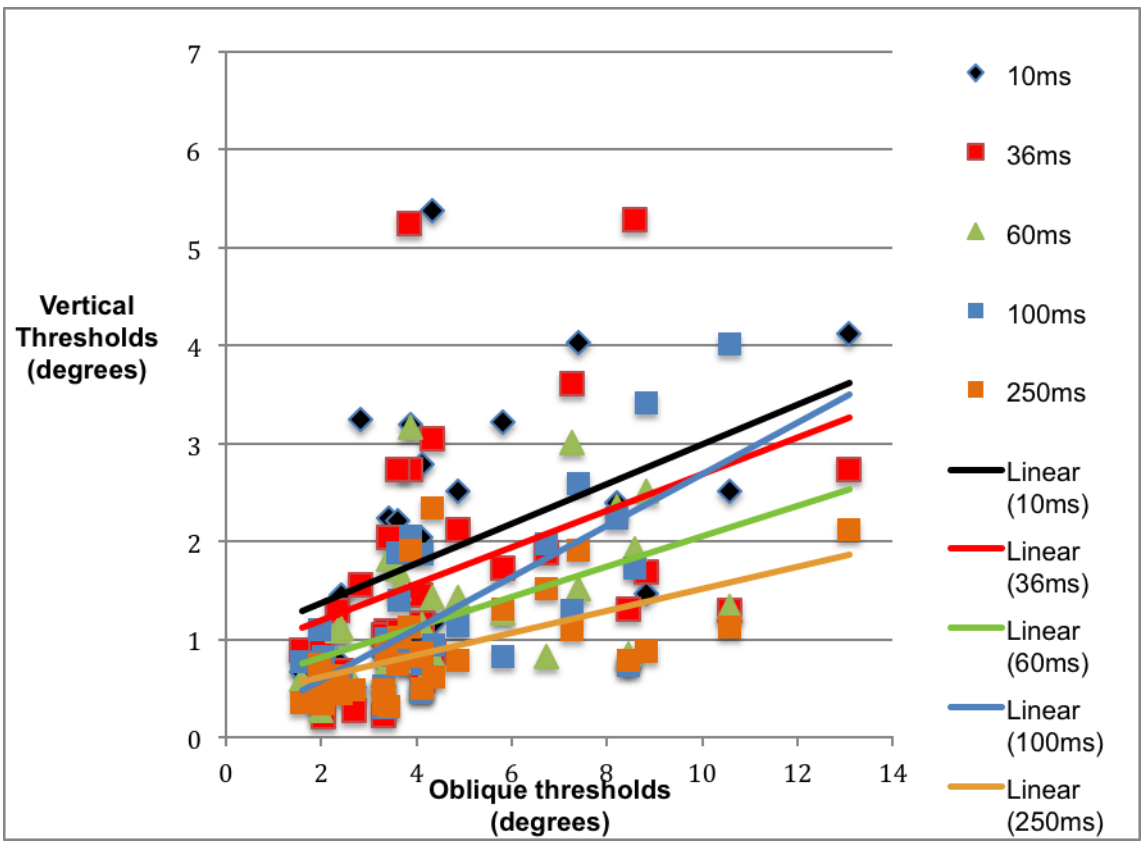


Figure 3.4– Vertical thresholds plotted as a function of the oblique thresholds with lines of best fit for each stimulus duration.

3.6 Discussion

This experiment has shown that decreasing the stimulus presentation time of the test patch increases participants' orientation discrimination thresholds. The results showed that the mean thresholds from the vertical 10ms condition were significantly higher than the 250ms condition indicating a decrease in performance as presentation time reduced.

There was also a very high correlation between the 10ms and 250ms vertical conditions suggesting that there were no ceiling effects present in the previous research by Edden et al (2009). Further, this and the other strong correlations between the conditions, suggest whilst the task got harder, participants performed very consistently across all of the conditions.

As the within subjects ANOVA demonstrated time had a main effect on threshold, it suggests the reduction in stimulus presentation time did indeed make the task harder. The mean threshold from the oblique condition was also significantly higher than the 10ms vertical condition, indicating that the oblique condition was still much harder than the hardest vertical condition administered. In line with this finding, there was a 30% dropout for the oblique condition compared to a max dropout of 17% for the vertical conditions (see Table 1). This seems high as Edden et al (2009) only reported a 7% dropout rate. This could simply be due to sample size as ours was ~3x larger in comparison. The dropout in this experiment demonstrates again that for some people this condition to simply too hard to complete. It would be interesting to isolate what makes this condition so hard for some people and not others thus it would be future use of this task could contain four conditions representing 'easy' and

‘hard’ for different orientations; 10ms and 250ms for both vertical and oblique orientations. This would provide more data regarding the difficulty of the task at oblique orientations and how this relates to other measures and levels of data loss.

One limitation of this study is the sample used was biased towards undergraduate students participating for course credit and were not particularly motivated to complete this task satisfactorily. This also made the average age of participants very young which could have also biased the results as orientation discrimination thresholds have been shown to increase with age (Betts, Sekular and Bennett, 2007). Future studies will need to include a much broader range of ages in order for this possible confound to not occur.

The results of this experiment demonstrated that the task does work and that neither celling effects were present. It also proved relatively easy to perform and easy to interpret. As such, it suggests it has significant potential for biomarker development in relation to psychiatric disorders such as schizophrenia.

3.7 Conclusion

This experiment suggests that no ceiling effects were present in the original research with the additional lack of floor effects found in this sample. This makes it a very promising task for future use and for biomarker development in healthy controls and in the patient population for which this is intended.

Chapter 4 - Investigation of the link between performance on the Tilt Illusion and Gamma oscillatory activity

4.1 Rationale

Performance on a Tilt Illusion task has been shown to negatively correlate with GABA levels in healthy controls when administered with the benzodiazepine Lorazepam. In this study we looked at the relationship between performance on the Tilt Illusion task and gamma oscillatory activity. Gamma measures will be used as a proxy measure for GABA levels in this experiment due to a previously reported positive correlation between the two measures.

4.2 Background

Visual perception relies heavily on context and has to adapt to successfully to changing surroundings. Gain control is a process that allows the visual system to adapt and boost responses to stimuli within a surrounding context by altering levels of inhibition and disinhibition to an orientation selective neuron (Clifford, 2014). Levels of gain control can be measured using methods targeting levels of surround suppression, whereby the presence of a surrounding stimulus influences the perception of a central target stimulus.

The Tilt Illusion (TI) measures the change in perceived orientation of a test patch in the presence of an oriented surround (See Figure 4.1). The size of this shift in perceived orientation is dependent on the orientation of the surround, with surround orientations between 0° and 45° producing a repulsion effect. This is known as the direct effect whereby the test target is perceived to rotate away from the orientation of the surround with the largest effect occurring at 15°

Figure 4.1 Tilt Illusion stimuli with surround

(Gibson, 1937). If, however, the orientation of the surround is between 45° and 90° then this produces an attraction effect whereby the orientation of the test grating is perceived to rotate towards the surround, with the largest effect occurring at 75° (Morant & Harris, 1965). This is known as the indirect effect.

When using this task in healthy controls, the direct effect in relation to vertical ranges from ~1.5-3°(Gibson, 1937; Gelbtuch et al., 1986; Wenderoth and Johnstone, 1988). This effect is very robust and can even occur when participants are prevented from consciously perceiving the surround, for example when performing a backward masking task where the presentation of one stimulus (masking stimulus) immediately after another brief target stimulus leads to a failure to consciously perceive the first stimulus (Clifford and Harris, 2005).

The direct effect is largest when there is no gap between the test and surround

gratings, with the size of the effect diminishing by nearly 50% when a gap of 1° is inserted between the two gratings (Wenderoth and Johnstone, 1988). This study also reported how altering the spatial frequency between test and surround gratings reduced the magnitude of the direct effect, again by nearly 50%, irrespective of whether there was a gap present. Finally the size of the surround grating greatly affected the direct effect. Reducing the size of the surround grating from 3.75° to 0.5° reduced the direct effect from 1.59° to 0.27° in this study. Thus, to obtain the best results the centre and surround used must have comparable characteristics with no gap present.

Gelbtuch et al (1986) investigated whether the size of the direct effect depended on GABAergic processes by administering Lorazepam, a benzodiazepine that potentiates activity at GABAa receptors, during a TI task. Their results showed a dose related increase in the size of the direct effect. Thus, the higher the dose of Lorazepam, the bigger the difference in perceived vertical between baseline and surround conditions. Interestingly this result was task specific, as Lorazepam had no effect on the results of a Tilt After Effect task, which is the same as the TI, but the centre and surround are presented sequentially. The effect was also medication specific, as Haloperidol, a dopamine D2 receptor antagonist, produced no dose related effect in the size of participants' direct effect in the TI task. This strongly suggests GABAergic activity influences the size of participants' direct effects calculated specifically from the TI task.

As discussed in the Introduction chapter of this thesis GABA is strongly linked to the production of synchronised oscillatory activity, primarily gamma activity.

Edden et al (2009) demonstrated this by showing a positive correlation between gamma frequency and occipital GABA levels in healthy controls. It could therefore be safe to assume that either measure could be used as a proxy for the other.

4.3 Aims and hypotheses

The purpose of this experiment was to refine the Tilt Illusion task in healthy controls and to GABAergic processes. Whilst the literature has focused on GABA levels, we used visually induced gamma frequency and amplitude as our measure due to ease and speed of testing but also due to its previously reported positive correlation with occipital GABA levels (Edden et al, 2009). This experiment therefore investigated whether gamma frequency and amplitude was correlated with the size of direct effects measured using the Tilt illusion. We are expecting to find a positive relationship between the size of the direct effect and visual gamma frequency and amplitude as measured by MEG.

4.4 Materials and Methods

4.4.1 Participants

Sixteen participants were recruited through the School of Psychology's Experimental Management System (EMS) and five opportunistically from CUBRIC, also part of the School of Psychology. The total sample was males n=6 (mean age 28 years) and females n=15 (mean age 21.6 years). All participants gave informed consent prior to testing.

4.4.2 Inclusion and exclusion criteria

The inclusion criterion was normal to corrected vision. Exclusion criteria

included a diagnosis of photosensitive epilepsy and having any metal in the body such as surgical pins or screws, pacemakers or implants.

4.4.3 Stimuli

The Tilt Illusion was controlled using DELPHI. The stimulus consisted of a central circularly-windowed grating of size 2 degrees, with a circularly-windowed grating surround of size 6 degrees, both 80% contrast (see Figure 4.1). When present, the orientation of the surround was kept constant at 15 degrees clockwise. In each trial the centre and/or surround was presented for 250ms. The psychometric functions and subsequent PSEs for each condition were calculated using MATLAB V.7.

Participants were seated 155.7cm away from the screen to produce the same visual angle as that produced in the MEG scanner. Their chin was placed on a chin rest to stabilise their head and standardise the distance from the screen between participants as best as possible. For the purpose of this study the room was kept in complete darkness for the duration of testing so participants could not see visible edges and corners, which may have influenced their responses. In addition, a board with a circular aperture was attached to the chin rest to block as many peripheral orientation cues as possible.

4.4.4 Procedure

For information on the methods used for the TI please see Chapter 2 section 2.2. Participants were instructed to rotate a flashing circular grating until they perceived the grating as being vertical by pressing the left or right buttons on the mouse. Flashing stimulus was used to avoid participants adapting to the

orientation of the stimulus. When they perceive the grating as vertical they were instructed to press the middle scroll button on the mouse. This was done four times to calculate a mean perceived vertical in degrees. The mean perceived vertical was used for the second part of the task as a midpoint for seven orientations spaced 1° three times either side. Participants were then presented with the same circular grating oriented randomly at one of the seven orientations calculated previously, for 250ms. They were instructed to respond whether they perceived it as being oriented to the right or left of vertical by pressing the corresponding button on the mouse. There were 10 trials per orientation totalling 70 trials per block with 6 blocks being administered. This task lasted approximately 30 minutes.

The output of interest from this task consisted of the calculated point of subjective equality (PSE), which in this case refers to when the participant can no longer discriminate between right and left and therefore is performing at chance level. To measure the size of the direct effect, the baseline ‘no surround’ PSE was subtracted from the surround PSE (see Figure 4.2). A positive PSE difference in this instance indicates a repulsive effect and reports the shift in terms of what direction the test circle would need to rotate in order to be at actual vertical.

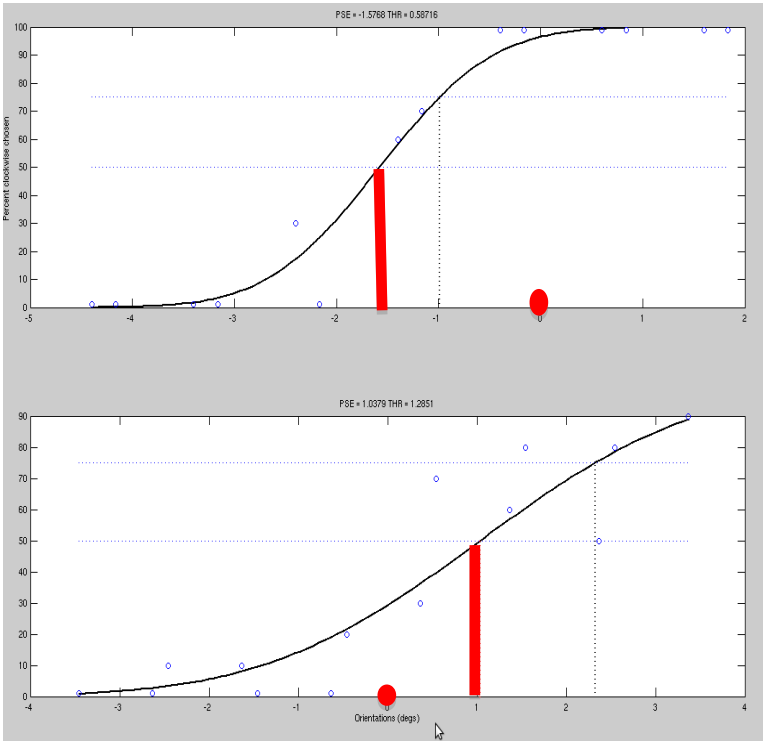


Figure 4.2 Example psychometric function. The top figure shows the no surround condition and the bottom figure shows the surround condition. Red line represents the PSE in each condition with the red circle representing actual vertical (0°). The difference between the two lines is the direct effect.

4.4.5 Magnetoencephalography

MEG recordings were gathered using a CTF-Omega 275-channel system sampled at 1200 Hz. Fiduciary markers were placed on the participant at fixed locations 1cm forward of each tragus and centrally between the eyes to enable MRI/MEG co-registration during analysis. Refer to Chapter 2 for in-depth discussion of MEG and MRI methodology.

The static visual stimulus presented consisted of a flashing full screen square-wave grating (100% contrast, 8° both horizontally and vertically, refresh rate 100Hz) with a central red fixation point (see Figure 4.3). This stimulus was presented for between 1500ms and 2000ms with a 3000ms resting period

between trials. Participants were instructed to press a button with their right index finger at stimulus offset. If they did not press the button at the correct time then they would receive feedback saying 'No response detected' to prompt correct performance. Each participant completed 1 block of 100 trials lasting approximately 8 minutes. Stimulus was presented on a Mitsubishi DiamondPro 2070 monitor at 1024 x 768 resolution at 100Hz. The monitor was situated approximately 208cm from the participant.

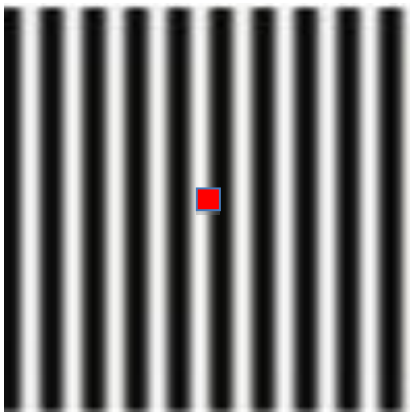


Figure 4.3 Static stimulus

Datasets were epoched into individual trials, with data excluded based on excessive noise or movement on a trial-by-trial basis. Synthetic aperture magnetometry (SAM) beamformer analysis was used to compare activity for 2s of baseline (-2 to 0s) and 2s of visual stimulation (0 to 2s). Pseudo t-statistics were used to compute any differences between baseline and stimulation activity. Volumetric SAM images were computed in six frequency bands, 0-20, 20-40, 30-70, 40-60, 30-80 and 80-130Hz, but for the purpose of this study analysis focused on the gamma band of 30-80Hz. The peak locations of gamma activity in each participants visual cortex were located and virtual sensors were constructed for these locations using covariance matrices bandpass filtered between 0 and 100 Hz. Time-frequency analysis of these

virtual sensors was conducted using a Hilbert transform method between 1 and 100Hz in 0.5Hz steps to produce time-frequency spectra. These spectra show peak gamma frequency and amplitude, expressed as percentage change from baseline. To determine the relationship between the psychophysics and the MEG measurements, the size of the direct effect was separately correlated with the transient gamma that occurred 0-0.3s after stimulus onset, and sustained gamma that occurred 0.3-0.8s after stimulus onset. Whilst previous research has focused on gamma frequency we will also investigate the relationship with gamma amplitude as, to my knowledge, this has not been looked at previously.

4.4.6 MEG: Gamma response quality control

Bootstrapping methods were applied to each dataset to be able to isolate and remove bad gamma datasets. For each iteration trials were resampled with replacement, and peak frequency was extracted from the power spectrum calculated as a percentage change from baseline. The bootstrap returned a distribution of 10000 bootstrapped peak frequencies, and the mode of this distribution was taken as reference. If at least half of the bootstrapped peak frequencies fell within 4hz (2hz either side) of the distribution mode, the dataset was classed as good. Bootstrapped mean peak frequencies and amplitudes, expressed as a percentage change from baseline, were extracted from the bootstrapped data for further analysis. Anyone who was excluded based on their sustained data (0.3-0.8s after stimulus onset) would also be excluded for their transient data (0-0.3s after stimulus onset) for that particular run.

4.5 Results

All MEG datasets passed quality control measures. All of the direct effects calculated were positive, indicating a shift in perception of vertical with the surround present (range 0.028-3.697degs). The mean PSE difference was 1.91degs (SD = 1.15degs). However, no significant relationship was found between transient gamma frequency and direct effect (r = 0.082, p=0.796), see Figure 4.4.

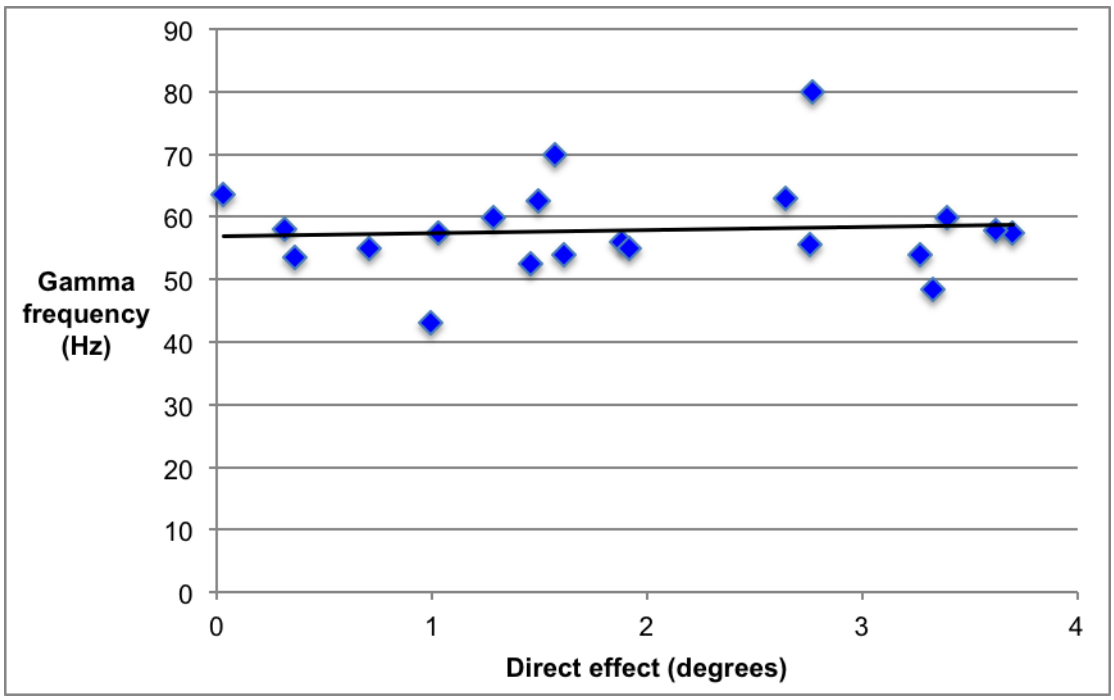


Figure 4.4 Scatter plot showing the relationship between PSE difference and transient gamma frequency with line of best fit.

No significant correlation was found between transient amplitude and PSE difference (r=0.177, p=0.632), see Figure 4.5.

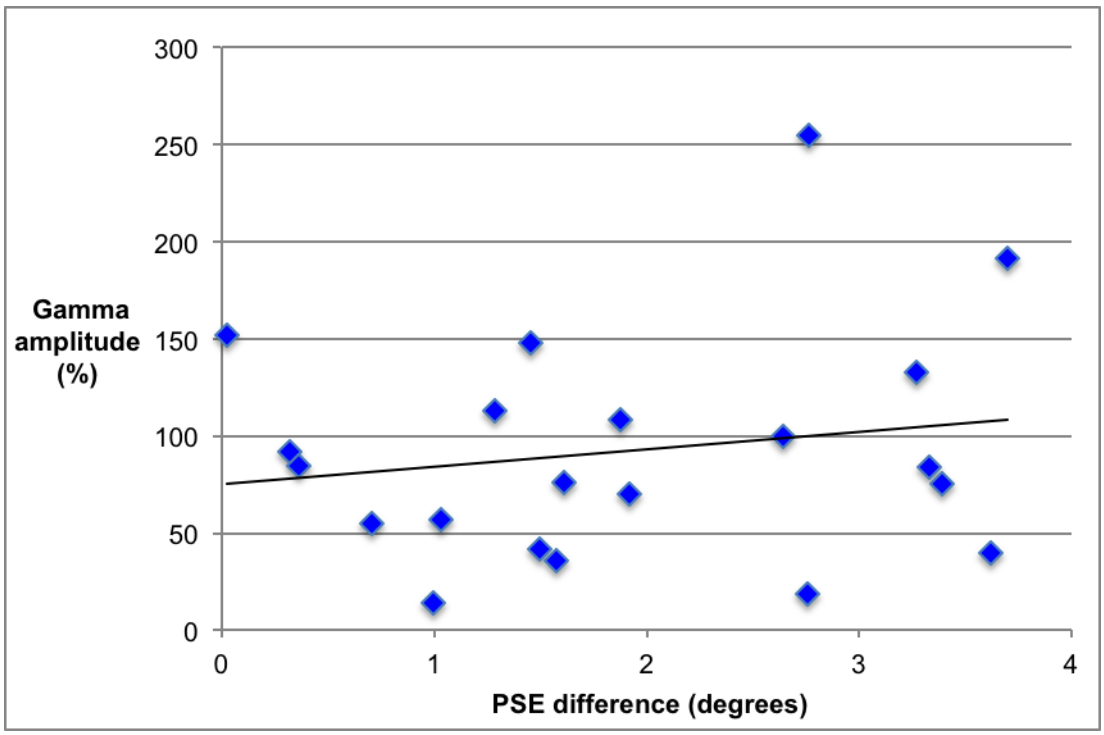


Figure 4.5 Scatter plot showing relationship between PSE difference and transient gamma amplitude with line of best fit

No significant relationship was found between sustained gamma frequency and PSE difference (r = 0.107, p=0.601), see Figure 4.6.

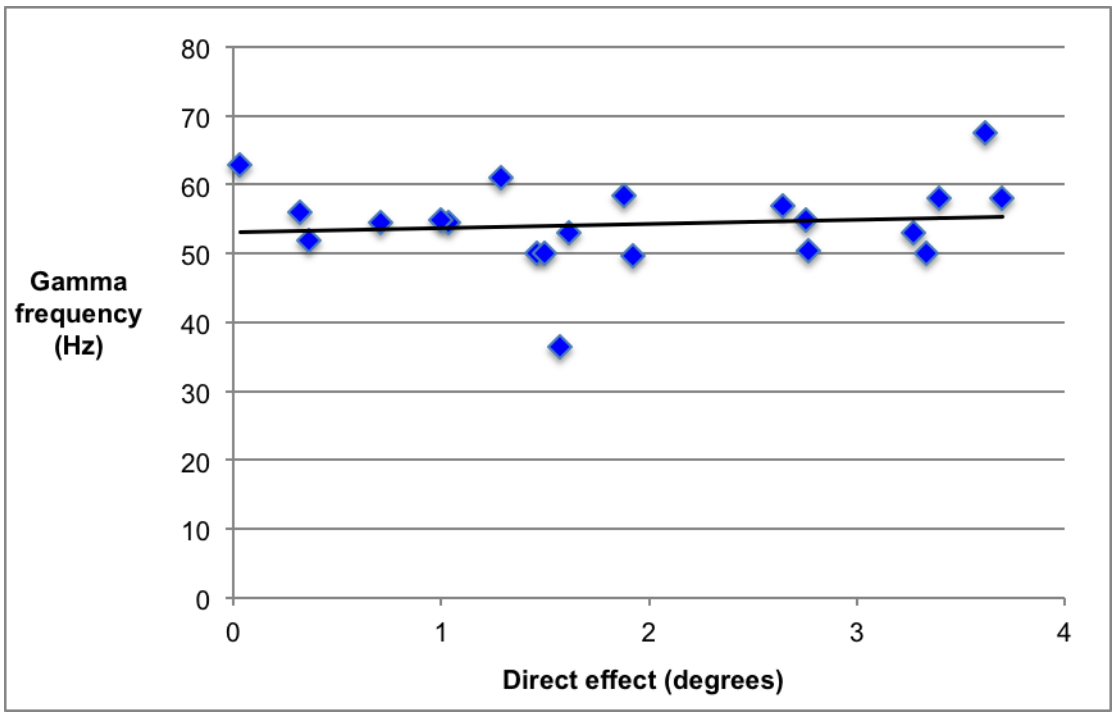


Figure 4.6 Scatter plot showing the relationship between PSE difference and sustained gamma frequency with line of best fit

No significant correlation was found between sustained gamma amplitude and PSE difference (r = 0.194, p=0.497), see Figure 4.7.

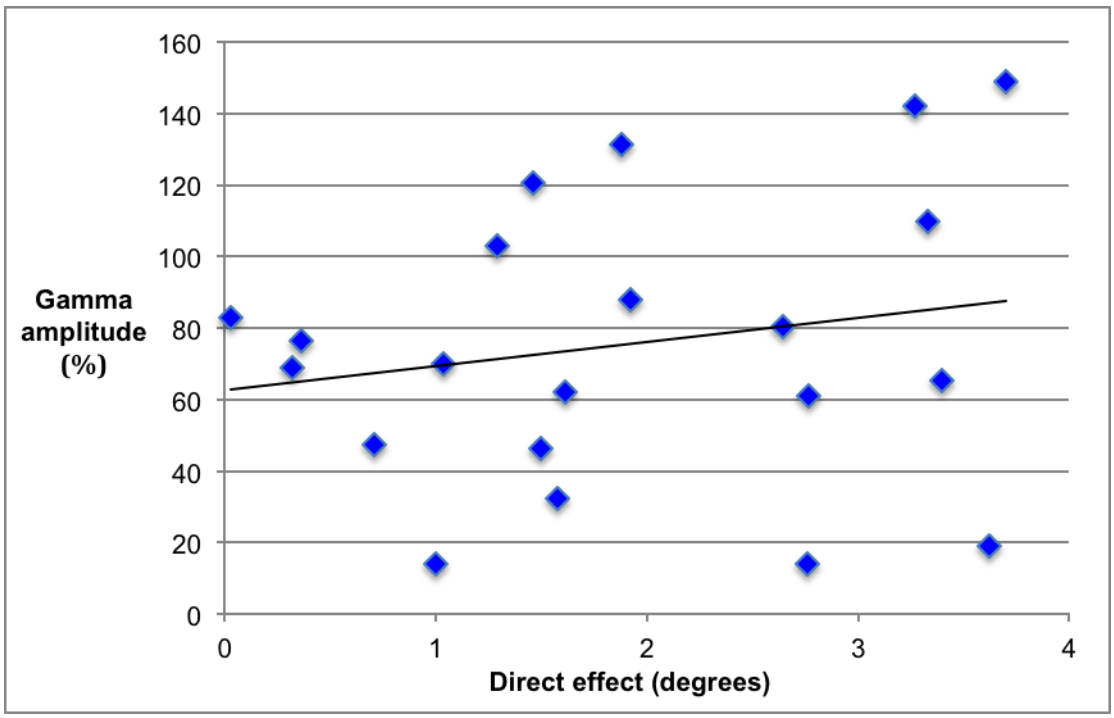


Figure 4.7 Scatter plot showing relationship between PSE difference and sustained gamma amplitude with line of best fit

Additional analyses using a repeated measures ANOVA showed no difference between the 3 no surround blocks (F(19) = 0.648, p=0.563) and the 3 surround blocks (F(19) = 0.459, p=0.352).

4.6 Discussion

This experiment showed there was no relationship between the psychophysical measurement of the direct effect and transient and sustained gamma frequency and amplitude. There are several possible explanations as to why these patterns of results occurred. One possible explanation is that the visual paradigm used to induce gamma oscillations and/or the Tilt Illusion task itself did not produce enough variability amongst the participants tested. This

however seems unlikely, as there was variability within the results for both spike and sustained gamma frequency and for the direct effect. This suggests that participants were not performing at ceiling and that the task was indeed a reasonable probe of individual variability within the sample tested. The measures have been shown to be reliable as analysis in this experiment suggested good test-retest reliability between the 3 blocks in each condition, although testing over different days would be needed to support this claim. Also visual gamma measured using the stimulus used in this thesis has been shown to provide highly repeatable results (Muthukumaraswamy et al., 2010). Both of these findings suggest the measures used were not responsible for the lack of relationship between the two measures.

A possible explanation for these results is that the positive correlation between gamma measures and GABA isn't as robust as first thought. For example recent work by Cousijn, Haegens, Wallis, Near, Stocks, Harrison et al., (2014) tried to replicate this relationship in 50 healthy controls and found no correlation between occipital GABA and gamma frequency. Although it should be mentioned that Gelbtuch and colleagues did not measure MRS GABA, which is the measure related to gamma frequency by Edden et al., (2009). Instead they inferred an increase in GABA from the administration of a GABA agonist, which increases the frequency of the opening of the chloride ion channel at the GABAa receptors. This does not necessarily mean there was an increase in in vivo GABA levels in the synapse but more an increase in activity at the synapse. This therefore could account for the lack of relationship between the direct effect and gamma activity in this sample. However, a relationship may still occur if measuring in-vivo GABA levels specifically, so future work using this

task should measure this and gamma activity in the same sample and relate this back to psychophysical measures.

The TI results did however validate the task itself, as all the PSE differences were positive meaning the repulsive effect occurred and performance was shifted when the surround was present. Additionally, our results were comparable with previous research (Gelbtuch et al, 1986)

Although the results here do not show a significant relationship between gamma and the direct effect this task is still worth pursuing, as there could still be group differences when investigating patient groups based on size of direct effects alone and a relationship could still be found when looking specifically at MRS GABA levels. Further to the possibility of using this paradigm in patient groups there are several advantages; it has high test-retest reliability, as additional analyses showed no significant differences between the separate blocks of each condition in terms of size of direct effect, it is easy to administer, and results are easy to interpret. As such, this task has significant potential for biomarker development in psychiatric disorders such as schizophrenia.

Finally, there were no differences in the three blocks of either the no surround or surround conditions suggesting this task is repeatable and validates the averaging of the three blocks of each condition in this and future studies.

4.7 Conclusion

This experiment showed no relationship between visual gamma frequency and PSE difference as measured by the Tilt Illusion. However, due to recent

research suggesting the positive relationship between gamma and GABA isn't as robust as first thought, further work in this thesis will still use this task and look at the relationship with both GABA levels and gamma frequency in both patients suffering with schizophrenia and age and gender matched healthy controls.

Chapter 5 - Estimating disease-related modulations in MRS GABA concentration in Schizophrenia

5.1 Rationale

Current antipsychotic medications derive their therapeutic action via the dopaminergic system in the brain. Although these types of antipsychotics are effective in reducing the positive symptoms associated with psychosis and schizophrenia, they have little or no effect on negative symptoms and cognitive deficits, which are arguably the most debilitating aspects of the disorder. Work on developing new antipsychotics is therefore targeted at other neurotransmitter systems that also interact with dopamine. The GABAergic system has become a prime candidate for reasons that are discussed in this chapter. This study will investigate whether there are any differences in GABA levels between healthy controls and patients with a DSM-IV diagnosis of schizophrenia and whether GABA relates to symptom severity in the patient group.

5.2 Background

Work in healthy controls has shown GABA to be related to several brain functions including motor decision speed (Sumner, Edden, Bompas, Evans & Singh, 2010), working memory performance using a prolonged delayed match-to-sample task (Michels, Martin, Klaver, Edden, Zelaya, Lythgoe, et al, 2012), BOLD response (Muthukumaraswamy, Edden, Jones, Swettenham and Singh 2009) and the generation of gamma oscillations, which are thought to play an important role in cognitive processes such as working memory (Michels, et al, 2012) and perceptual processes such as orientation discrimination (Edden et al., 2009).

The idea of a reduction in GABA levels or GABAergic properties in patients with schizophrenia is widely supported. The most consistently reported finding is a reduction in the 67-kDa isoform of the GABA synthesising enzyme glutamic acid decarboxylase (GAD67); Curley et al., 2011; Guidotti et al., 2000). This reduction in GAD67 has been localised to parvalbumin-containing GABAergic interneurons, which has been confirmed in post-mortem studies in the motor and visual cortex (Hashimoto et al., 2008), hippocampus (Knable et al., 2004) as well as the dorsolateral prefrontal cortex (Akbarian et al., 1995). Mouse models using GAD67 knockout mice (mice that express no GAD67), supported these findings by showing that these mice produced only 7% of the GABA concentrations of wild type mice thereby strongly suggesting that a reduction in the expression of this enzyme would lead to a reduction in GABA levels overall (Asada et al, 1997).

GABA levels are known to be altered in the frontal region in the brains of those with schizophrenia (Rowland et al., 2015). Post-mortem studies have identified GABAergic chandelier cells to be dysfunctional with changes in their form and functionality, and up to a 40% decrease in the axon terminal density shown in the frontal brain of patients with schizophrenia (Lewis, Cho, Carter, Eklund, Forster, Kelly, et al, 2008; Pierri, Chaudry, Woo and Lewis, 2014). Chandelier cells also known to act via GABAa receptors specifically which is relevant to schizophrenia as GABAa receptors have been shown to modulate spatial and temporal memory performance (Mohler, 2009), something known to be impaired in patients affected by schizophrenia (Dreher, Banquet, Allilaire, Paillère-Martinot, Dubois & Burnod, 2001).

Based on the evidence that GABA plays an important role in cognition and perception in healthy controls, it is not surprising that is has emerged as an area of interest for research into psychiatric disorders. The symptoms associated with schizophrenia in particular manifest as a marked reduction in cognitive abilities, spanning all cognitive domains but most severe in relation to working memory (Chen et al, 2014). Abnormalities are also seen in perceptual processes such as surround suppression psychophysical tasks using motion, orientation and size related stimuli, (Yoon et al., 2010) and motion detection (Chen, Palafox, Nakayama, Levy, Matthysse and Holzman, 1999). Additionally, impairments in face perception, especially in relation to identifying emotions have been identified (Salem, Kring, & Kerr, 1996).

When looking at GABA levels in vivo, results for patients are mixed with some reporting an increase in GABA in chronic patients (Öngür et al., 2010) while others report a reduction (Yoon et al, 2010; Marsman et al., 2014). One possible reason for these differences could be the level and variety of antipsychotic medications being taken by the patients. Evidence from clinical studies suggests that antipsychotic medication could affect the GABAergic system either at the receptor level or by altering GABA concentrations (Wassef et al., 2003). Atypical antipsychotics clozapine and olanzapine reduce hippocampal and temporal cortex GABAa receptor density in rats by approximately two-thirds whereas there is a reduction of only 10-20% with older, ‘typical’ antipsychotics haloperidol or chlorpromazine suggesting that antipsychotics are not uniform in their effects on GABAa receptors (Farnbach-Pralong, Bradbury, Copolov & Dean, 1998). These results relate to the acute use of antipsychotics and in contrast for longer term use (more than 7 days),

clozapine and haloperidol produced increased GABA turnover rates across 4 different brain regions that was consistent between the two medications, suggesting no difference in their effect on GABA when used in this way (Marco, Mao, Revuelta, Peralta & Costa, 1978). This uniform effect on GABA after chronic use is reassuring, as patients tend to be on a variety of different antipsychotics. However, it does suggest that antipsychotics can influence GABAergic mechanisms.

More recently Rowland and colleagues (2015) demonstrated that after converting antipsychotic medication dosages to their chlorpromazine equivalents, its inclusion as a covariate did not change their finding of a reduction in GABA levels in patients with schizophrenic illness, suggesting chronic medication use did not explain the difference in MRS GABA levels in this sample.

The brain region of analysis for GABA measurement is an important factor to consider although the global reduction seen in GAD 67 expression across brain areas in patients would suggest this is not the case Curley et al 2011; Guidotti, et al, 2000). In contrast using MRS GABA measurement Kegeles and colleagues (2012) found conflicting results from two voxels positioned in the dorsolateral and medial prefrontal cortex with an increase in GABA levels in unmedicated patients in the medial prefrontal region but not in the dorsolateral prefrontal cortex. This study also reported no differences in GABA levels between healthy controls and medicated patients in both voxels raising the possibility that medication, within these brain regions, may reduce GABA in patients.

Age is also an important factor to consider when measuring MRS GABA as levels decline with advancing age in healthy controls (Gao, Edden, Li, Puts, Wang, Liu et al, 2013), with this decline appearing to be more rapid in patients with schizophrenia (Rowland et al, 2015). It would be logical to assume that patients who are medicated would tend to be older than those who are unmedicated and this could account for the conflicting results identified by Kegeles and colleagues. It would therefore be highly recommended to age match controls to patients as best as possible in future research.

5.3 Aims

As previous studies in patients tend to focus on one brain region with mixed results, this study will compare GABA levels between patients with a DSM-IV diagnosis of schizophrenia and healthy age and gender matched controls in two separate voxels positioned in the occipital/visual cortex and sensorimotor cortex (see Figure 5.1). These areas have been shown to produce repeatable and sensitive results suggesting these are reliable voxels from which to collect GABA data (Evans, McGonigle & Edden, 2009). We predict there will be a significant reduction in GABA levels in patients compared to controls in both voxels. This study will also look at the relationship between GABA and anti-psychotic medication as well as symptom severity.

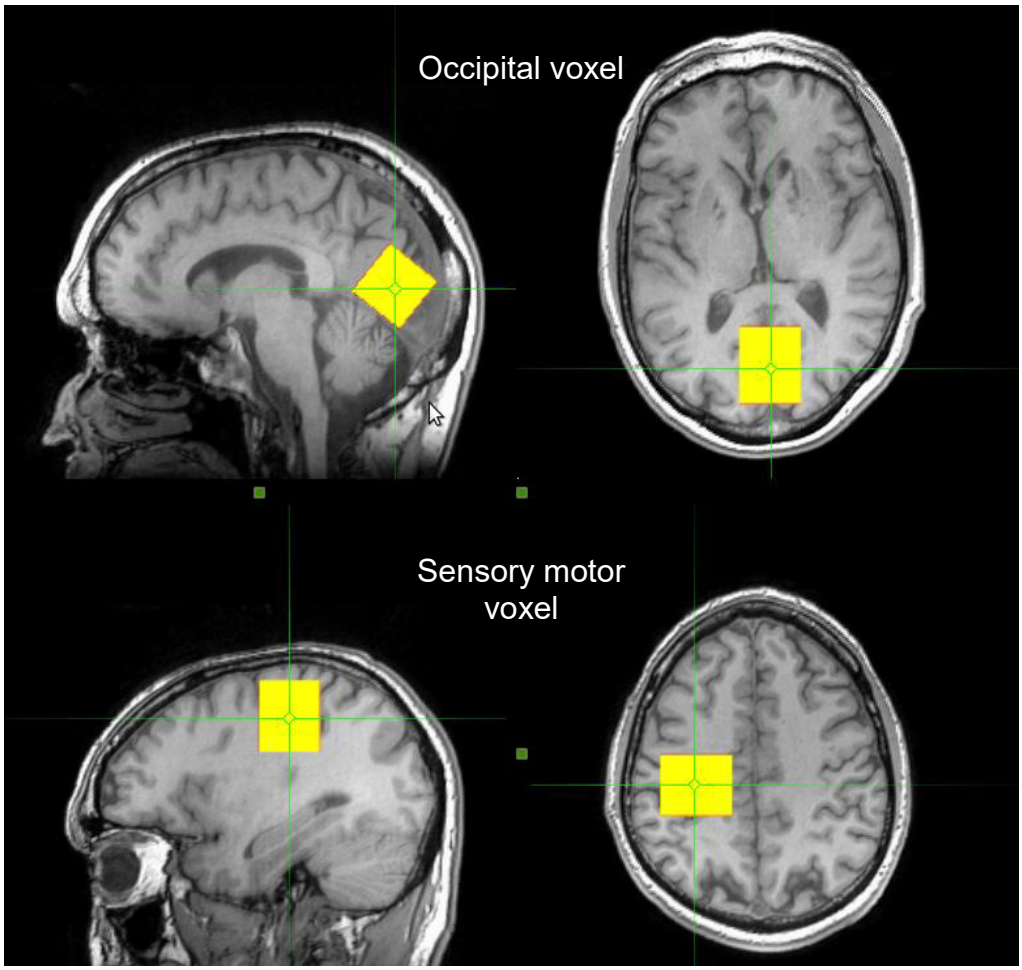


Figure 5.1 Voxel placements used during MRS scans

5.4 Methods

5.4.1 Participant sample and recruitment

Participants consisted of 28 patients all of whom had a DSM-IV primary diagnosis of schizophrenia (8 female, mean age males = 44.20 (SD = 8.59) years, females = 45.88 (SD = 7.97) years) and 30 control participants (11 female, mean age males = 42.37 (SD = 9.98) years, females = 40.636 (SD = 10.15) years). Patients were recruited through a previous Cognition in Psychosis study also conducted at Cardiff University (Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Please see Methods section for more details on patient recruitment. Controls were recruited primarily

through an advert placed on the Cardiff University Noticeboard system and opportunistically from CUBRIC, Cardiff University. This study was approved by the NHS ethics Board and also the Cardiff University School of Psychology Ethics Board.

5.4.2 Inclusion and exclusion criteria

Inclusion criteria for the patient group consisted of a DSM-IV diagnosis of schizophrenia as determined through the SCAN interview, being aged between 16-75 years, having English as a first language and having normal or corrected vision. Inclusion criteria for healthy controls included being between 16-75 years of age, having English as a first language and normal or corrected vision. We ensured that all participants had sufficient capacity to give their informed consent to participate. All participants were screened for current drug or alcohol abuse. If they did not report current abusive behaviour they were asked to refrain from any alcohol or drug use 48 hours prior to testing.

Exclusion criteria for all participants were a diagnosis of epilepsy, any severe neurological event such as head injury or stroke, any metal present in their bodies or the current use of medication in relation to affective disorders. Appendix 1 shows the patients' current medication in the form of olanzapine equivalents. Additionally, healthy controls were excluded if they or if a first-degree relative had any previous mental health diagnosis of an affective or psychotic disorder.

5.4.3 MRS and analyses

MR data were acquired using a 3 Tesla General Electric Signa HDx scanner

with an eight-channel receive-only head RF coil (Medical Devices). Foam padding was used to minimise head movement during scanning. A 5-minute 3D T1-weighted structural scan (TR/TE/TI = 7.8/3.0/450ms, flip angle=20° FOV = 256*192*172mm, 1mm isotropic resolution) was obtained for each participant for voxel placement during analysis.

Two 3x3x3cm^3 voxels located in the occipital lobe and sensory motor area were scanned using the MEGA-PRESS method (Mescher et al., 1998) to acquire GABA-edited spectra for those areas (TE = 68, TR=1800 with a 20ms Gaussian editing pulse applied at 1.9ppm in alternate ON/OFF scans). The occipital voxel was placed medially in the occipital lobe, positioned as posteriorly as possible but still preventing inclusion of the sagittal sinus. The sensorimotor region was defined as being centered on the “hand knob” area of the precentral gyrus, and in line with the upper surface of the brain in the sagittal plane (Yousry, Schmid, Alkadhi, Schmidt, Peraud, Buettner et al., 1997). See Figure 5.1 for voxel placements. Acquisition time was ten minutes thirty-four seconds for each scan.

Quantification of GABA was calculated using Gannet 1.0 toolkit (Edden, Puts, Harris, Barker, & Evans, 2014) and in house MATLAB scripts. Spectra were excluded if the fit error was above 10% with those remaining being corrected for gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) tissue proportions in relation to water.

5.4.4 Questionnaires and diagnostic interviews

The Beck Depression Inventory – II (BDI-II (Beck, Steer, and Brown (1996)) and the Altman Self-Rating Mania Scale (ASRM (Altman, Hedeker, Peterson &

Davis (1997)) were used to get a current measure of depression and mania symptoms respectively. The MINI International Neuropsychiatric Interview (Sheehan, Lecrubier, Sheehan, Morim, Janavs, Weiller, et al, 1998) was administered to the control group to gather information on any current or past experiences of neuropsychiatric symptoms and disorders. If any were present that participant would be excluded. Only the psychosis section was administered to the patients and only in relation to the previous two weeks in order to get a current measure of psychosis. The author had been trained to administer the MINI prior to testing and had previous experience in administering the Schedule for Clinical Assessment in Neuropsychiatry (SCAN) in-depth clinical interview. This information then informed the ratings on the Scale of the Assessment of Positive Symptoms (SAPS (Andreason, N (1984)) and the Scale of the Assessment of Negative Symptoms (SANS (Andreason, N (1984)) which rate the positive and negative symptoms associated with a diagnosis of schizophrenia. Details of antipsychotic medication were recorded including daily dosages which were standardised across patients using Olanzapine equivalents as calculated by Gardner, Murphy, O,Donnell, Centorinno & Baldessarini (2010)

5.5 Results

5.5.1 Demographics

Age and gender did not differ significantly between groups ($t(57) = -1.119$, $p = 0.268$ and $t(57) = 0.812$, $p = 0.420$, respectively). In examining the case and control groups by gender, no significant differences were found between patients and controls in terms of age; males ($t(37) = -0.625$, $p=0.536$) and females ($t(18) = -0.969$, $p= 0.345$).

5.5.2 Occipital GABA analysis

Five patients (4 males and 1 female) and three controls (2 males and 1 female) were excluded from Occipital analysis due to excessive movement or a fit error of >10%. After exclusion of these participants age and gender still did not significantly differ between groups ($t(49) = -2.546$, $p = 0.067$ and $t(49) = -1.016$, $p = 0.398$ respectively).

Occipital GABA levels significantly differed between groups ($t(49)=2.389$, $p= 0.021$) with the patient group having lower GABA levels compared to controls (see Figure 5.2). To confirm that these results were not driven by differences in tissue ratios in the occipital voxel between groups, we compared the levels of CSF, gray matter and white matter. Independent t-tests showed no significant differences in the amount of each tissue between groups; t(49), 0.462, p=0.646, t(49), 0.097, p=0.923 and t(49), -0.160, p=0.874 respectively. We used Pearson correlation to demonstrate that age did not significantly correlate with occipital GABA in the sample as a whole ($r = -0.189$, $p=0.184$), age accounting for a small percentage of the variation of GABA levels ($R^2=0.0357$). Similarly there were no effects of age on GABA levels within each group; controls ($r=-0.292$, $p=0.131$) or cases ($r=0.073$, $p=0.753$ see Figure 5.3). For example spectra output from Gannet see Figure 5.4. Appendix 2 shows the mean GABA levels and standard errors for each group and the sample as a whole for both voxels.

Finally, Pearson correlation demonstrated no significant correlation between GABA and medication (defined as olanzapine equivalents) within the patient group ($r = -0.148$, $p=0.510$) with medication accounting for 2.19% of the variance in GABA ($R^2 = 0.0219$). Please see Figure 5.5.

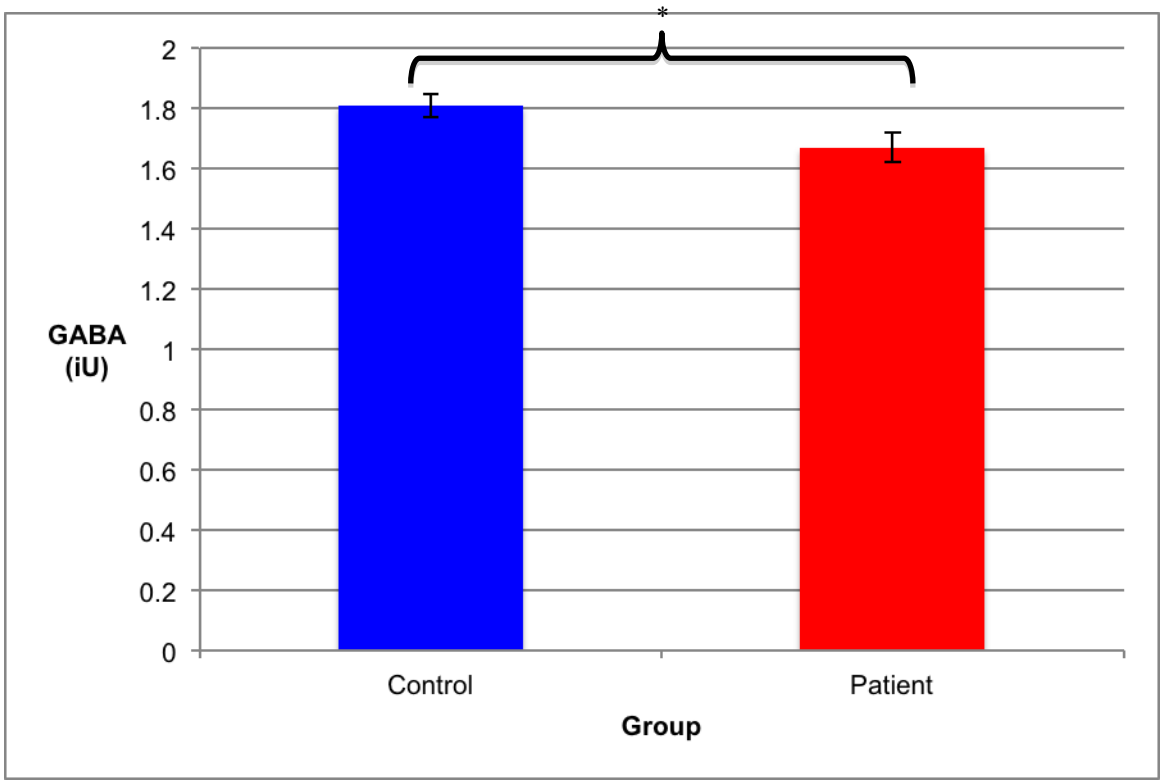


Figure 5.2 Mean occipital GABA levels for the Patient and Control groups. Error bars show +/- 1 Standard error of the mean. * = p<0.05

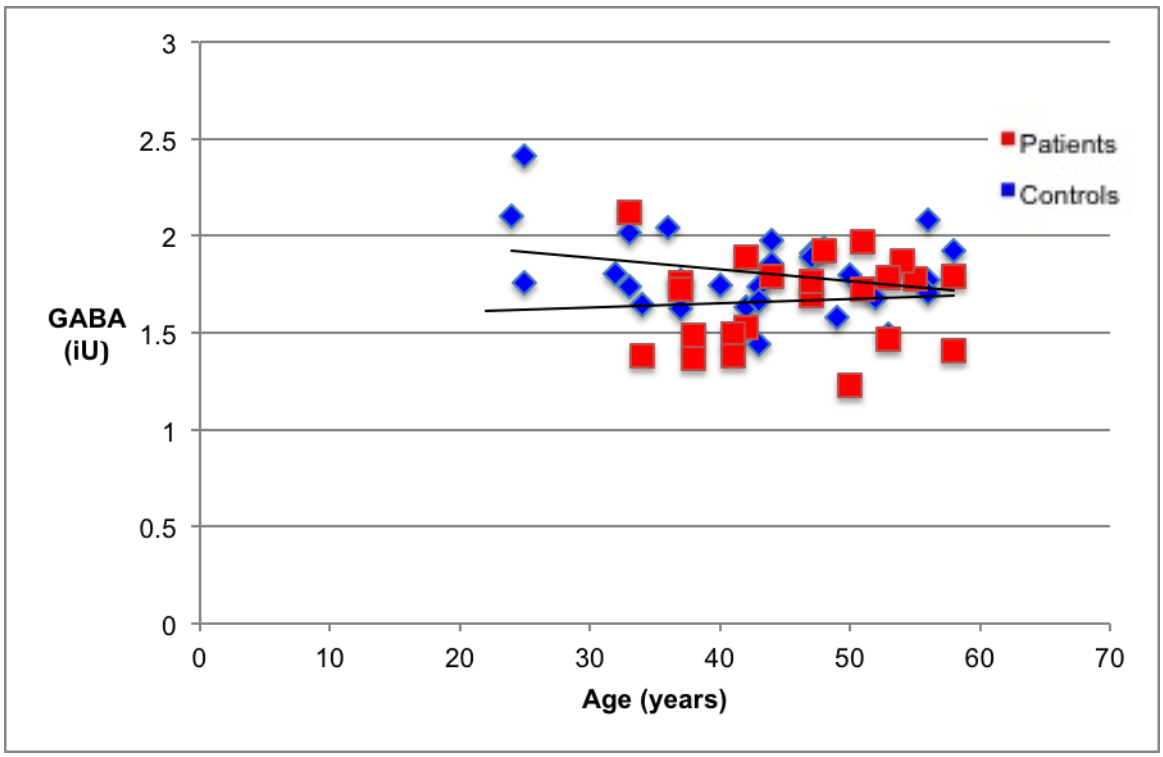


Figure 5.3. Correlation of age and occipital GABA levels for both patient and control groups

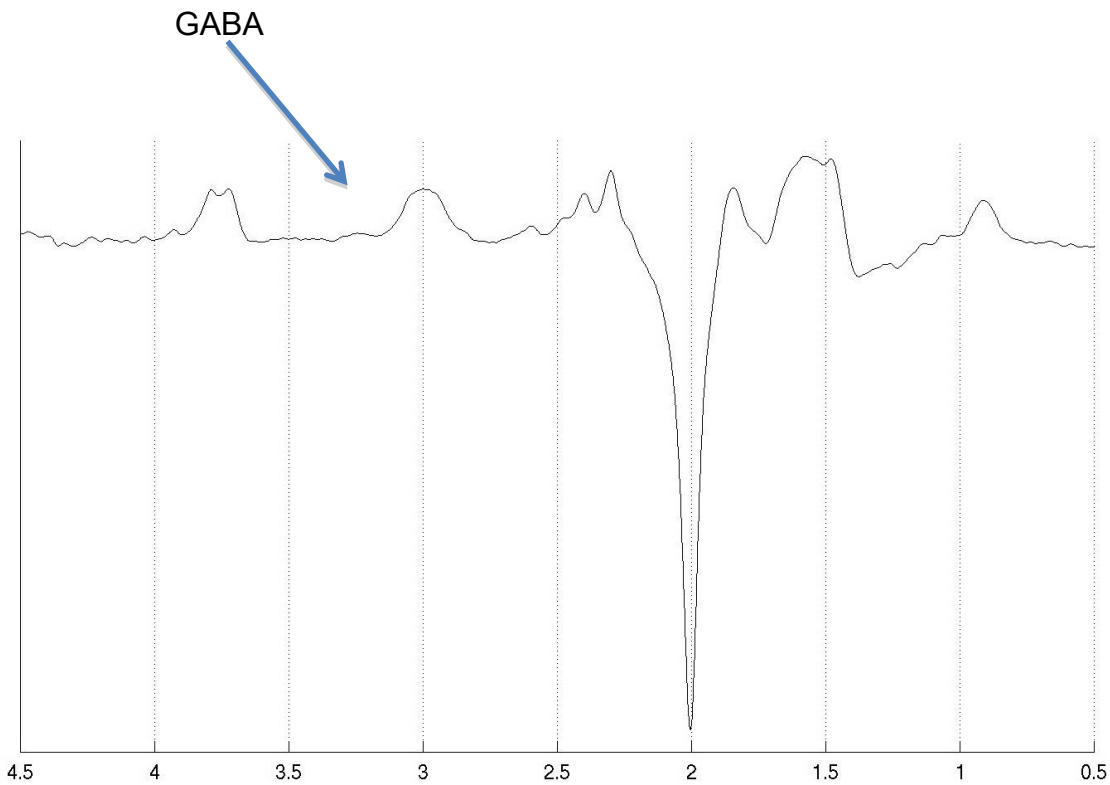


Figure 5.4 Example Gannet spectra output

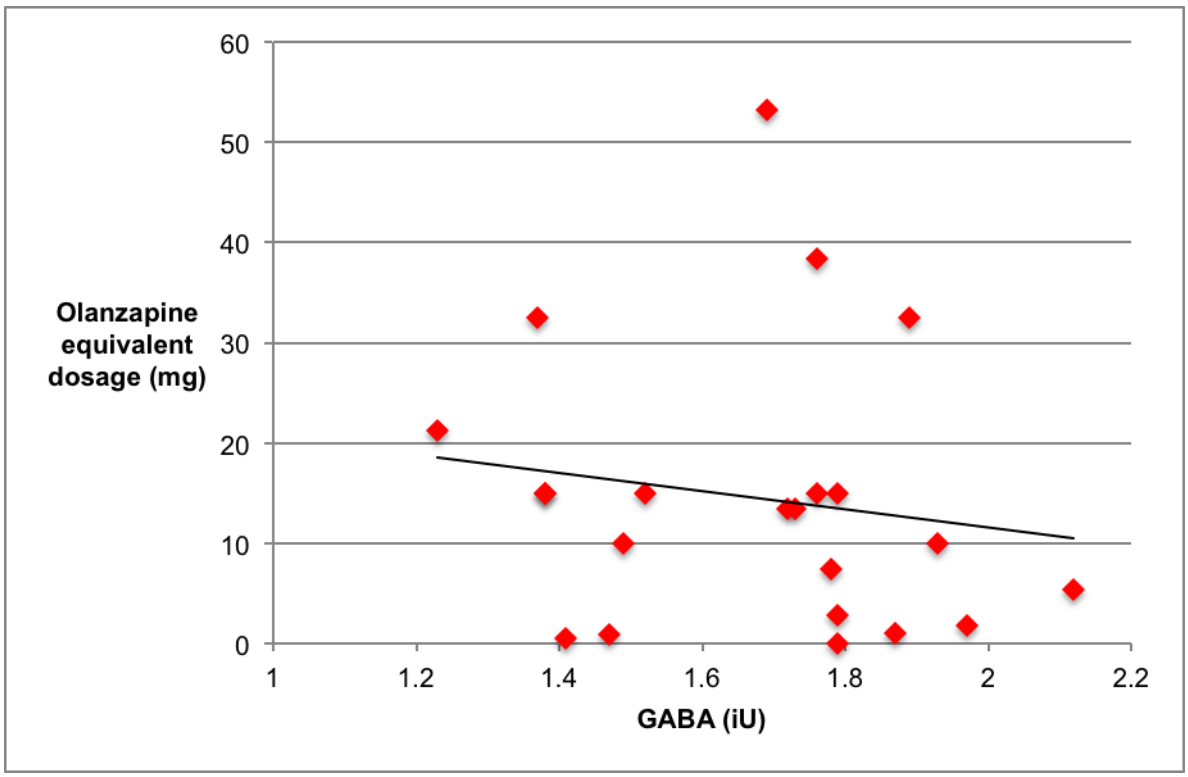


Figure 5.5 Correlation of occipital GABA and olanzapine equivalent anti-psychotic dosages for cases.

5.5.3 Sensory motor GABA analysis

Six patients and two controls were excluded from sensory motor analysis due to excessive movement or a fit error of >10%. Sensory motor GABA levels were not significantly different between cases and controls (t(49), -1.236, p=0.222), see Figure 5.6). Pearson correlations showed GABA levels from both voxels did not correlate within the sample as a whole (r = -0.048, p=0.748) or at group level; controls (r = -0.079, p=0.689) and cases (r = 0.162, p=0.495). A paired t-test showed sensory motor GABA levels were significantly lower than occipital within the sample as a whole (t(47)=10.966, p = <.001) and within the control group (t(27) = 10.809, p=<.001) and cases (t(19) = 5.465, p=<.001) independently (see Figure 5.7).

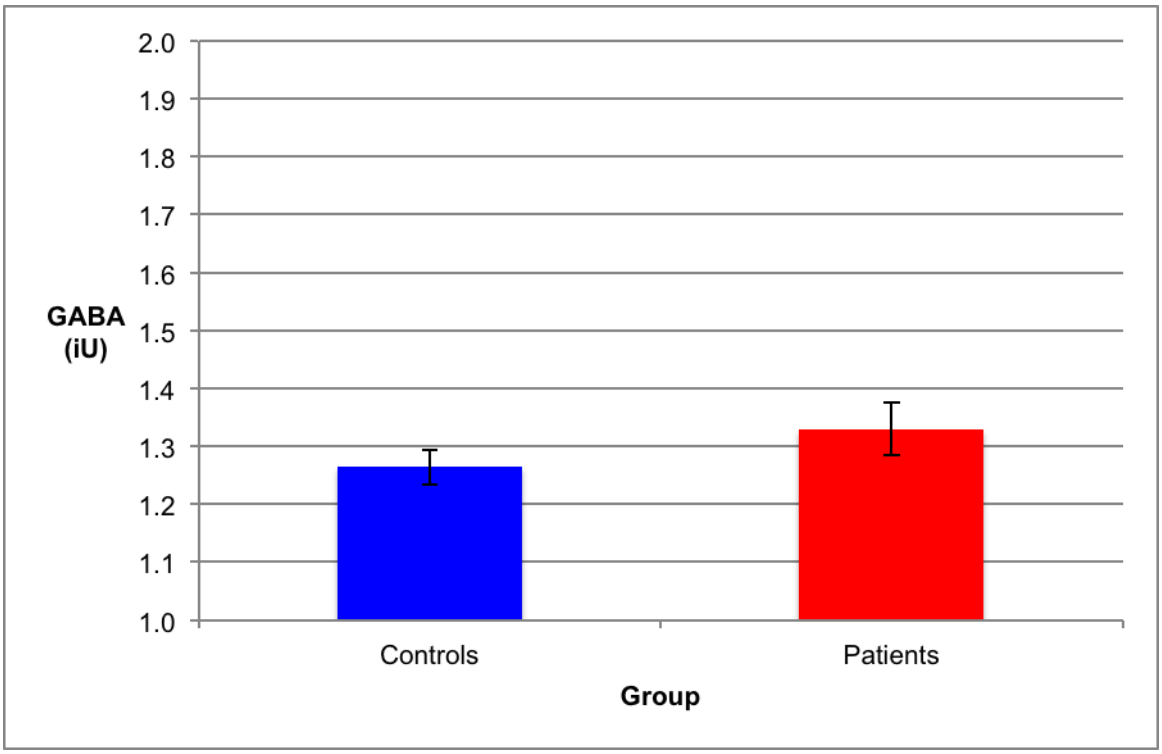


Figure 5.6 Mean sensory motor GABA levels for Control and Patient groups. Error bars show +/- 1 standard error of the mean

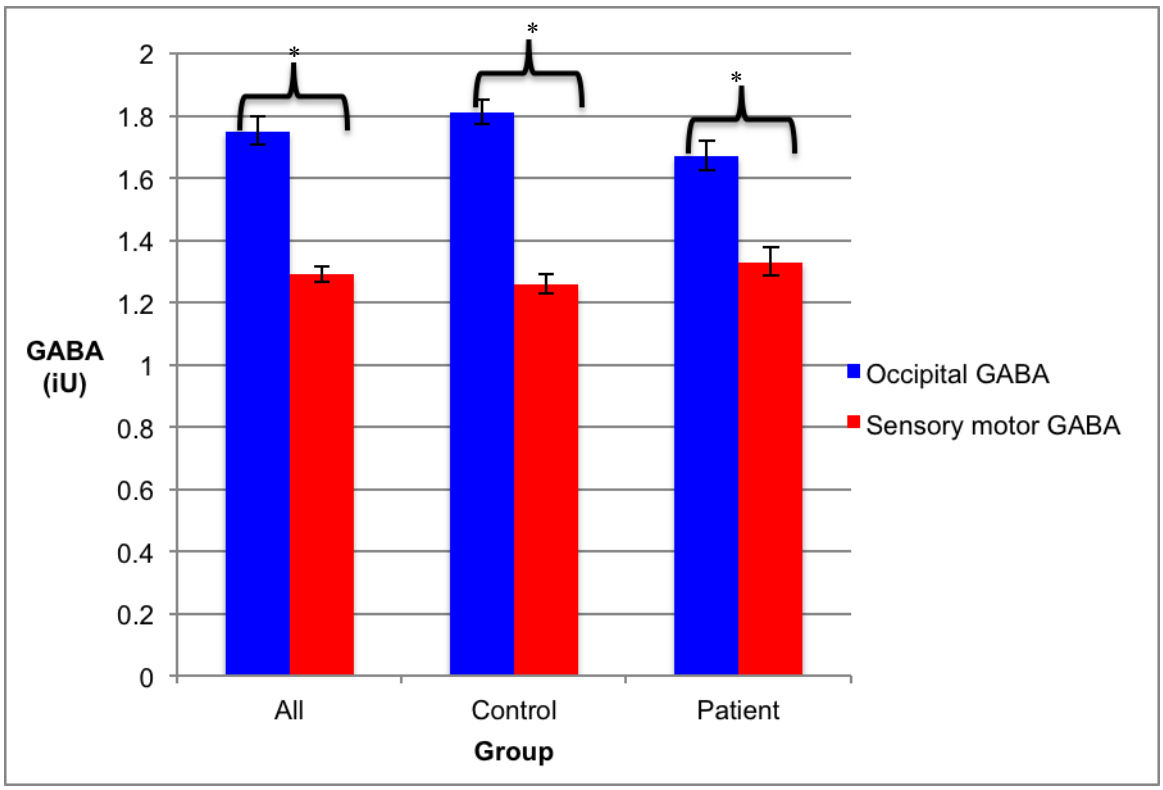


Figure 5.7 Comparison of mean occipital and sensory motor GABA levels for the sample as a whole and each group separately. Error bars show +/- 1 standard error of the mean. *=p<0.001

5.5.4 Symptom measures

There were highly significant group differences in BDI scores (t(36) = -4.202, p=<.001). For the controls, twenty-nine had scores classed as minimal depression (scores 0-13) with one classed as having mild depression (score 14-19). For the patients, thirteen were classed as having minimal depression, six as mild depression, six as moderate depression (score 20-28) and two as having severe depression (score 29-63). There were no between group differences in the Altman mania scores (t(55) =-0.501, p=0.618). See Figure 5.8. For the controls, twenty-four had scores classed as no manic symptoms, five classed as hypomanic and one classed as manic. For the patients, twenty-two were classed as having no manic symptoms, five classed as hypomanic and one classed as manic. SAPS and SANS scores were 0 for all control

participants and consequently no analyses were performed between groups for these measures.

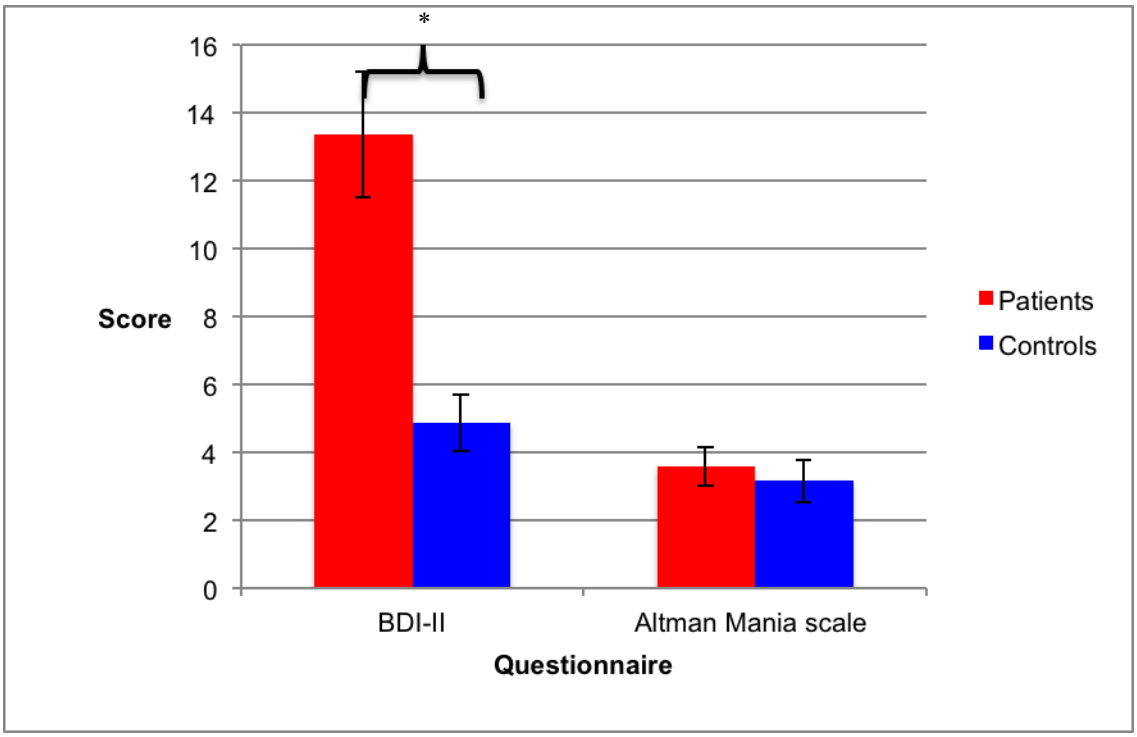


Figure 5.8 Comparison of mean scores for the BDI-II and Altman Mania scale between groups. Error bars show +/- 1 standard error of the mean. * = p<0.001

Within the Patient group, occipital GABA negatively correlated with SAPS scores (r = -0.429, p=0.041) and SAPS and BDI scores were positively correlated with each other (r = 0.474, p = 0.013). None of the symptom measures correlated with sensory motor GABA levels. See Table 5.1 for a breakdown of the symptom measures.

	BDI-II		**Altman Mania scale**		**SAPS**		**SANS**	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Mean	13.37	4.86	3.59	3.16	3.43	0	3.96	0
SD	9.58	4.56	2.89	3.45	15.14	0	7.66	0
Range	0-29	0-14	0-12	0-14	0-12	0	1-12	0

Table 5.1 Breakdown of symptom measure scores

5.6 Discussion

This experiment demonstrates that GABA levels are altered in specific brain regions in patients with a DSM-IV diagnosis of schizophrenia compared to healthy age and gender matched controls. There were significant between group differences in occipital GABA, with patients having decreased levels compared to controls, but no such differences in sensory motor GABA levels. This result is not unexpected, as other studies have reported differing results when quantifying GABA in different voxels in the same patient sample (Zegeles et al, 2012). GABA levels did not correlate between voxels at the group or sample level and differed between voxels with sensory motor levels being lower than occipital, which replicates previous findings (Evans et al, 2010).

One possible reason for the reduction in only one of the voxels measured is that as the patients used in this sample were chronic patients, it could the case that the reduction in occipital GABA is a biomarker of chronic illness. Longitudinal studies using first episode patients scanned in several different voxels over time could indicate if indeed occipital GABA is a biomarker of chronic illness.

Olanzapine equivalents of anti-psychotic medications did not correlate with occipital GABA suggesting that this was not the primary source of variation in GABA within the patient group and thus not responsible for the observed between group difference in GABA levels. This supports the work by Rowland et al (2015) that a showed using chlorpromazine equivalent, reduced GABA in the frontal region of the brain was not related to anti-psychotic medication. Longitudinal studies comparing the same patients before and after anti-psychotic use in several voxels could answer the question as to what extent

GABA is affected by the disease and the medication. The method of standardising the medication could have affected the results, however, we also calculated chlorpromazine equivalents following the methods of Gardner and colleagues (2010) and similarly these dosages showed no association with GABA levels in either voxel.

However, this sample size is still relatively small and we were not able to look at the effects of different classes of antipsychotics. The class of antipsychotics could produce differing effects on GABAergic processes (Farnbach-Pralong et al., 1998) and it might be the case that the reduction found in this experiment was medication specific. In addition olanzapine equivalents can only produce a standardised measure for current usage and does not take into account lifetime exposure to antipsychotics. Glial production and hypertrophy of the cerebral cortex has been related to antipsychotic exposure over time and are thought to play a regulatory role in adjusting neurotransmitter levels (Selemon, Lidow and Goldman-Rakic, 1999), potentially impacting on the GABAergic system. Whilst olanzapine equivalents is a frequently used measure for standardizing anti-psychotic treatment, there is a potential underlying confound of lifetime exposure that should be measured and accounted for, although there is still uncertainty about how this can reliably be done.

As there was only a reduction in GABA in one of the voxels measured, this lends further supports to antipsychotic medication not being responsible for the reduction in occipital GABA within the patient group. If medication were contributing to the reduction in GABA then it is unlikely this would be region specific and one would expect reductions across the brain, including the

sensory motor area. However, future studies will need larger sample sizes of participants taking different classes of antipsychotics as again, this assumption could be based on the effects of one class of antipsychotics.

The previous research showing discrepancies in GABA level findings across studies were measuring from different cohorts in relation to exposure and response to antipsychotics, which makes them harder to compare to this study. Elevations were reported in unmedicated patients (Kegeles et al, 2012) whereas reductions are most widely reported in chronic medicated patients (Yoon et al, 2010; Kegeles et al, 2012; Rowland et al 2015). As this experiment used chronic medicated patients it supports previous research. However, it is very hard to disentangle medication effects from illness effects, as it is difficult to measure every possible combination of patient and medication exposure. For example it is extremely difficult to test antipsychotic-naïve first episode and chronic patients. Further, the samples used for research such as this utilise a subset of patients who are willing to participate and who are responding well to treatment. Therefore they could be seen as atypical and representing only a small proportion of patients. The current ongoing MRC-funded SPRING study is trying to remedy this problem by testing different cohorts of patients with different levels of exposure and response to antipsychotics.

Age did not correlate with occipital GABA and based on the group as a whole only accounted for 3.57% of the variation in GABA levels. There was a negative trend within the control group with GABA decreasing with age but this was not the case within the patient group, an unexpected finding as previous research

had shown the decline in GABA with age to be accentuated in patients with schizophrenia (Rowland et al, 2015).

A potential problem with comparing these results to those reported by Rowland et al (2015) is that their participant sample was not limited to patients with a diagnosis of schizophrenia but also included those with schizoaffective disorder. Thus, their lack of findings between GABA and medication could potentially be due to the initial disease state of the participant and additional medications. This does not appear to be the case in this sample as there was no difference between mania scores as measured by the Altman Mania Scale. This is encouraging as it supports the patients' original diagnosis of schizophrenia and does not suggest symptoms related to schizoaffective disorder. Therefore, this sample appears to be much better suited to answering the question of whether GABA levels differ in patients with a diagnosis of schizophrenia.

Patients reported experiencing much higher levels of depression, with 29% currently experiencing moderate to severe levels of depression, as measured by the BDI-II compared to the controls group. This is in line with previous findings showing approximately 20% of chronic schizophrenia patients experience moderate to severe depression using the BDI-II also (Chemerinski, Bowie, Anderson & Harvey, 2008).

GABA levels obtained in this experiment were macromolecule contaminated, also referred to as GABA+, which could potentially affect the reliability of the result. A recent study by Mikkelsen, Singh, Sumner & Edden (2015) has shown macromolecule suppressed GABA, GABA', is no less repeatable than GABA+

suggesting using this method is sufficient to produce reliable GABA results. However, GABA' does produce a purer measure of GABA so should be used if possible in future studies.

Whilst tissue correction was performed on the MRS data in this experiment, this is not an exact science. Voxel tissue segmentation is calculated by segmenting a mask of the voxel on the participants FSPGR image and approximating the contribution of CSF, white and gray-matter to that voxel. As it is an approximation, by definition the values calculated might not be correct which could impact on the GABA levels measured. GABA concentrations have previously been found to be higher in gray matter compared to white matter (Choi, Lee, Merkle and Shen, 2006; Bhattacharyya, Phillips, Stone, and Lowe, 2011) and so therefore between participant variability could be affected by the methods used for tissue correction.

Another potential limitation of this experiment is the use of water as our reference molecule where creatine has been shown to be highly reliable as a reference (Bogner, Gruber, Doelken, Stadlbauer, Ganslandt, Boettcher, et al, 2009). However, MRS research has shown creatine levels to be disrupted in this patient group (Öngür et al., 2009). Therefore, the use of creatine as a reference molecule in schizophrenia MRS research is problematic unless creatine levels have been shown to be comparable between groups beforehand.

5.7 Conclusion

This study demonstrated a reduction in occipital GABA but no difference in sensory motor GABA in patients with schizophrenia compared to controls. The inconsistency between voxels is not surprising, as other studies have also found differing results depending on the voxels investigated in this patient group.

Chapter 6 - Novel low-level oscillatory markers of cortical excitation/inhibition in Schizophrenia: An investigation of disease sensitivity and optimal task parameters

6.1 Rationale

Gamma oscillations have previously been linked to connectivity between brain regions and levels of cognition in healthy control and patient groups. Therefore, gamma measures have become of interest in research investigating possible biomarkers for reduced cognition in patient groups. Visually induced gamma band oscillations are reliably measured using static grating stimuli. Patients with a diagnosis of schizophrenia have reduced gamma activity in terms of frequency and amplitude using visual tasks. Moving and attentionally engaging stimuli have been shown to increase both visually induced gamma frequency and amplitude in healthy controls compared to the use of static stimuli. This type of task has not yet been used in patients and due to their reported reductions in gamma oscillatory activity, it would be interesting to investigate whether this increase occurs for patients also. This experiment could also identify a more sensitive measure of gamma activity in general, but one that is also better suited to patients.

6.2 Background

Synchronised activity of neural populations can give rise to oscillations of different frequencies, recorded in local field potentials. Neural oscillations across different frequency bands are thought to impact on specific brain functions including perception and cognition (Engel, Fries & Singer, 2001; Palva

and Palva, 2007). Gamma band (30-150Hz) synchronisation has recently become of interest as it has been implicated in many important brain functions, including visual change detection in primates (Womelsdorf, Fries, Mitra & Desimone, 2006) as well as visuomotor control (Aoki, Fetz, Shupe, Lettich & Ojemann, 1999; Aoki, Fetz, Shupe, Lettich & Ojemann, 2001), working memory (Howard, Rizzuto, Caplan, Madsen, Lisman, Aschenbrenner-Scheibe et al 2003; Roux, Wibral, Mohr, Singer & Uhlhaas, 2012), and attentional processes (Sokolov, Pavlova, Lutzenberger & Birbaumer, 2004; Tallon-Baudry, Bertrand, Hénaff, Isnard & Fisher, 2005).

Gamma band synchronisation occurs through a temporal coding scheme whereby the firing of groups of neurons within different areas of the visual cortex become synchronised, allowing communication between these areas. Supporting this idea Gray, Konig, Engel & Singer (1989) showed gamma oscillations synchronise intercolumnar input in the cat visual cortex thus showing neuronal populations in different areas of the visual cortex would fire in phase during a cycle of gamma frequency oscillations. It was proposed that these different neuronal populations could be conveying different attributes of the visual scene and by firing in phase would help form a unified scene.

It is only relatively recently that non-phase locked (induced) gamma band activity has been successfully recorded in humans (Tallon-Baudry, Bertrand, Delpuech, & Pernier, 1996; Tallon-Baudry, Bertrand, Delpuech, & Pernier, 1997). Visually induced gamma activity has now become one of the most studied areas in relation to neural oscillations as simple high contrast static

grating stimuli have been shown to elicit consistent and repeatable occipital gamma-band oscillations in healthy controls (Muthukumaraswamy et al., 2010). As well as being repeatable, among all frequency bands, they are the most susceptible to stimulus changes with increased contrast associated with increasing gamma amplitude (Henrie and Shapley, 2005), annulus grating stimuli and larger full screen stimuli leading to increased gamma power compared to square-wave grating stimuli (Muthukumaraswamy and Singh, 2013). A reduction in amplitude is also seen when using a plaid grating stimuli (Hermes et al., 2014).

Moving grating stimuli have been shown to increase both visual gamma amplitude and frequency, whilst additionally producing results with a clearer induced sustained effect in both macaque monkey (Ray and Maunsell, 2010) and healthy human controls at approximately 65-70Hz (Swettenham et al., 2009; Muthukumaraswamy and Singh, 2013).

For both of these types of moving stimulus the biggest increase in induced-frequency was in participants who had the lowest induced-frequencies when presented with the static stimuli (Swettenham et al., 2009). This suggests that some level of ceiling effect may be occurring, as most participants' gamma frequency response increased to 65-70Hz when presented with a moving stimulus, irrespective of their response when presented with a static stimulus.

An attentionally engaging stimulus also produces an increase in gamma frequency (Vidal et al., 2006) and when used in conjunction with contracting a radial moving stimuli produces similar results with a clear sustained effect

between 65-70Hz with an additional separate gamma band at around 40Hz for a subset of participants (Hoogenboom et al., 2006). However, the evidence for an attention related increase in gamma amplitude/frequency is rather contradictory: In a recent paper, Koelewijn, Rich, Muthukumaraswamy and Singh (2013) showed that visuo-spatial attention increased broadband higher-frequency gamma, but did not enhance narrow-band gamma in the 30-70Hz, matching similar findings in macaque monkey (Chalk, Herrero, Gieselmann, Delicato, Gotthardt and Thiele, 2010).

Given the association between gamma oscillations and cognitive functioning, as well as their relation with brain connectivity, the potential of gamma oscillations in psychiatric research has begun to be explored and exploited. Among the core symptoms of schizophrenia are impairments, in cognitive functioning. In addition a prominent theory of the disorder's pathophysiology is the dysconnectivity hypothesis, which states that the symptoms of the illness stem from a dysfunction in communication between different brain regions (Friston, 1999). Thus, research into schizophrenia and neural oscillations is focusing more on the gamma band due to its implication in information transfer between different brain regions and cognitive functioning.

Inhibitory parvalbumin+ interneurons are known to work cyclically with excitatory pyramidal cells, producing extracellular field potentials that manifest as brain oscillations, in particular gamma band oscillations (Gonzalez-Burgos et al, 2010; Williams and Boksa, 2010; Buzsaki and Wang, 2012). Therefore gamma may be disrupted due to changes in GABAergic function, glutamatergic

input to interneurons and membrane properties suggestive of fundamental problems within underlying synaptic circuitry impairments.

Given the above, it is possible that modulation of either gamma amplitude or frequency may be predicted to occur in those with schizophrenia. This disruption of oscillatory activity may also be reflective of dysfunction across the cortex, including within regions not traditionally associated with schizophrenia, such as the primary visual cortex.

In support of this, a reduction in gamma oscillatory activity has been widely reported in patients with schizophrenia with recent work showing a general reduction in induced gamma amplitude both at rest and during working memory tasks (Haenschel et al, 2009; Chen, et al, 2014) and backward-masking tasks (Wynn et al., 2014). Additional reductions in high gamma power (60-120Hz) have been recorded in the visual cortex during the perception of Mooney faces (Grutzner et al., 2013) and during auditory stimulation (Hamm et al., 2011).

As it has been demonstrated that visual gamma can be reproducibly and robustly induced with low-level grating stimuli (Swettenham et al., 2009; Muthukuraswamy and Singh, 2013), it would seem to be a sensible strategy to use this type of task to probe synaptic function in schizophrenia – to date, this has not been demonstrated and is the key aim of this experimental chapter. A subsidiary aim is to investigate the optimum task design to robustly probe these measures in both patients and healthy controls.

6.2.1 Medication Confounds

Antipsychotic medication has been shown to modulate gamma activity

irrespective of disease state. Rats under the influence of both first and second-generation anti-psychotics showed inhibited hippocampal gamma power due to the medications interaction with D3 dopamine receptors (Schulz et al., 2012). However, the same pattern of results have been found irrespective of medication state, as unmedicated first episode patients have demonstrated impaired cognitive control related gamma activity and also reduced evoked prefrontal gamma-band responses during an auditory task (Minzenberg et al., 2010; Gallinat et al., 2004 respectively). It could be the case that both antipsychotic medication and the disorder itself independently affect oscillatory mechanisms and that the combination amplifies the effect.

6.2.2 Dependence on brain structure

Brain structure has been implicated as a mediating factor in the disruption of neural oscillations and has been related to gamma frequency with the surface areas of V1 and V2 positively correlating with visually induced gamma in healthy controls (Schwarzkopf et al., 2012). Cortical thickness in area V1, peak transient and peak sustained gamma frequency have been negatively correlated with age (Gaetz et al., 2011). Reductions in cortical volume, thickness and surface area in patients have also been reported (Rimol et al., 2011; Edgar et al., 2014) thus, an assumption could be, based on the above control data, that patients would have reduced peak transient and sustained gamma frequency. In spite of this assumption, there has been no research investigating whether differences in induced visual gamma activity are mediated by structural differences in patients with schizophrenia.

6.2.3 Gamma and GABA

GABAergic inhibition has been found to be inextricably linked to gamma-band activity as the interplay between inhibitory GABA interneurons and excitatory pyramidal cells is both necessary and sufficient for the production of gamma oscillations (Traub, Whittington, Stanford & Jefferys, 1996); Wang and Buzsáki, 1996). In addition, several connected GABAergic inhibitory neuron networks are major generators of gamma oscillations. In humans, bulk GABA concentration can also be estimated using MRS and GABA concentration has been shown to correlate with gamma frequency in healthy controls (Muthukumaraswamy et al., 2009; Edden et al, 2009) and in patients for gamma frequency and amplitude (Chen et al, 2014). However, a recent MRS/MEG study in a large cohort of healthy controls failed to find such a relationship (Cousijn et al., 2014)

6.3 Aims

Our key aim is to investigate whether low-level induced visual gamma oscillations within primary visual cortex provide a sensitive marker of the underlying pathology in schizophrenia.

Our principal hypothesis is that we will see a reduction in both transient and sustained gamma amplitude and frequency in the patient group compared to healthy age and gender matched controls. We also investigate whether patients demonstrate similar increases in visually induced gamma amplitude and frequency when using a moving radial grating task compared to a static grating task. Using repeated presentation of the moving radial task, we will also investigate the repeatability of this task in both patients and controls over time.

In addition we will also investigate the relationship between occipital GABA and visually induced gamma-band oscillations to try to replicate previously found correlations between the two measures in healthy controls and patients with schizophrenia. Additional analyses will examine the relationship between positive, negative and affective symptom measures and gamma oscillation outcomes.

Due to the association between cortical structure and gamma oscillatory activity and the reduction in cortical structure measures in schizophrenia cases, this experiment will also gather information on brain structure and correlate with the MEG and MRS data. This will demonstrate how much of the variance within the MEG and MRS data can be attributed to structural variables.

Finally, olanzapine equivalents will be used to standardise antipsychotic medication dosage within the patient group (Gardner et al 2010). Again these will be correlated with the gamma measures to demonstrate how much of the variance can be attributed to medication effects.

6.4 Methods

6.4.1 Participant sample and recruitment

Participants consisted of 31 control participants (12 female, mean age males = 42.37 (SD = 9.98) years, females = 41.17 (SD = 12.04) years) and 28 patients all of whom had a DSM-IV primary diagnosis of schizophrenia (8 female, mean age males = 44.20 (SD = 8.59) years, females = 45.88 (SD = 7.97) years. For more details on how the patients were recruited and diagnosed please see Methods chapter. Controls were recruited primarily though an advert placed on

the Cardiff University Noticeboard system and opportunistically at CUBRIC, Cardiff University. Patients were recruited through a parent genetics study also conducted at Cardiff University. Please see Chapter 2 for more details regarding patient recruitment. Patients were age and gender matched to controls on a pairwise basis.

6.4.2 Inclusion and exclusion criteria

Inclusion criteria for the patient group consisted of a DSM-IV diagnosis of schizophrenia as determined through the SCAN interview, being aged between 16-75 years, having English as a first language and having normal or corrected vision. Inclusion criteria for healthy controls included being between 16-75 years of age, having English as a first language and normal or corrected vision. We ensured that all participants had sufficient capacity to give their informed consent to participate. All participants were screened for current drug or alcohol abuse. If they did not report current abusive behaviour they were asked to refrain from any alcohol or drug use 48 hours prior to testing.

Exclusion criteria for all participants were a diagnosis of epilepsy, any severe neurological event such as head injury or stroke and any metal present in their bodies. Additionally healthy controls were excluded if they or if a first-degree relative had any previous mental health diagnosis of an affective or psychotic disorder.

6.4.3 Magnetoencephalography

MEG recordings were gathered using a CTF-Omega 275-channel system sampled at 1200 Hz. Fiduciary markers were placed on the participant at fixed

locations 1cm forward of each tragus and centrally between the eyes to enable MRI/MEG co-registration during analysis.

Each participant undertook four experimental visual gamma experiments in each measurement session:

6.4.4 Experiment 1: Static Visual Grating

The static visual stimulus used was identical to that used in Chapter 4 (section 4.4.5).

6.4.5 Experiments 2, 3 and 4: Moving Radial Grating Stimulus

This experiment is based on the paradigm of Hoogenboom et al.,2006. Each trial of the moving radial stimulus started with the presentation of a fixation point (diameter 0.5 °). After 500 ms, the fixation point contrast was reduced by 40 %, to warn participants of the upcoming trial. After 1500 ms, the fixation point was replaced by a centrally presented, circular sine wave grating (diameter 5°, spatial frequency 2 cpd and maximum contrast). The circular grating drifted inwards toward the fixation point and the speed of this contraction could increase (velocity step at 2.2 deg/s) at any time between 50 and 3000 ms after stimulus onset (see Figure 2). The participant was instructed to press a button with their right index finger as soon as they noticed the speed of the contraction increase. This response would have to be within 500 ms of the increase in speed to be recorded as correct. Ten percent of the trials were catch trials in which no increase in speed occurred. The stimulus stopped after a response was given, or in catch trials 3000 ms after stimulus onset. Stimulus offset was followed by a resting period of 1000 ms in which subjects were given visual

feedback of either ‘OK’, ‘early’ or ‘late’ depending on their response. Each participant completed 3 blocks of 80 trials lasting approximately 25 minutes. This stimulus was controlled by Presentation software (Neurobehavioural Systems) and presented using a projector located outside of the magnetically shielded room, which was then projected back onto a screen within the room approximately 60cm from the participants’ eyes at a nominal refresh rate of 60Hz. Please see Figure 6.1 for task outline.

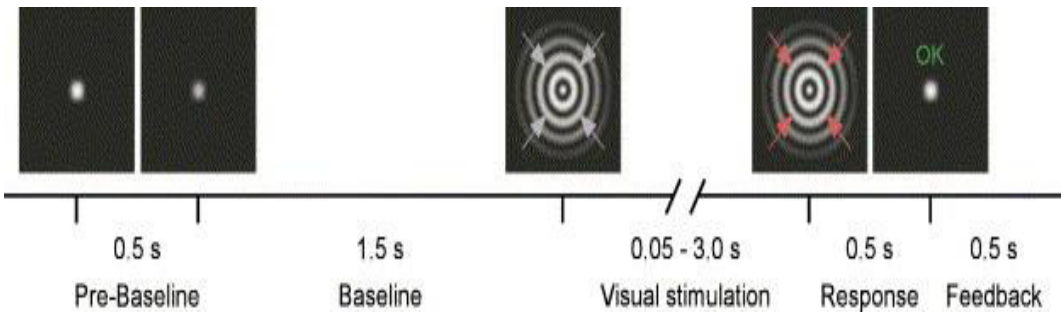


Figure 6.1 Task outline for the moving radial stimulus (obtained from Hoogenboom et al., 2006)

Datasets were epoched into individual trials and were excluded based on visual inspection of the data on a trial-by-trial basis. Synthetic aperture magnetometry (SAM) beamformer analysis was used to compare activity for 2s of baseline (-2 to 0s) and 2s of visual stimulation (0 to 2s). Pseudo t-statistics were used to compute any differences between baseline and stimulation activity. Volumetric SAM images were computed in six frequency bands, 0-20, 20-40, 30-70, 40-60, 30-80 and 80-130Hz, but for the purpose of this study analysis focused on the gamma band 30-80Hz. The peak locations of gamma activity in each participant’s visual cortex were located and virtual sensors were constructed for these locations using covariance matrices bandpass filtered between 0 and 100 Hz. Time-frequency analysis of these virtual sensors were conducted using

method based on the Hilbert transform (Swettenham et al., 2009) between 1 and 100Hz in 0.5Hz steps to produce time-frequency spectra in which the responses at each time/frequency point were expressed as mean percentage change from baseline.

6.4.6 MEG: Gamma response quality control

Quality control methods used are identical to those in Chapter 4 (Methods section 4.4.6)

6.4.7 Magnetic resonance imaging and spectroscopy

MRI and MRS methods are identical to those used in Chapter 5 section 5.4

6.4.8 Freesurfer structural analysis

FreeSurfer analsysis was used to estimate the cortical surface area, thickness and volume of V1 for each participant from their FSPGR scan. FreeSurfer is an automated technique that first identifies the calcarine sulcus and then uses prior knowledge of the cortical curvature to estimate the location of V1 (Hinds, Rajendran, Polimeni, Augustinack, Wiggins, Wald et al., 2008). These structural estimates were calculated for each hemisphere separately.

6.4.9 Questionnaires and diagnostic interviews

The questionnaires and antipsychotics equivalents used were identical to those used in Chapter 5 (section 5.4.4).

6.5 Results

6.5.1 Quality control measures

Quality control measures were performed on the data to isolate and remove 'bad' gamma datasets. Table 6.1 shows the numbers of participants remaining after quality control measures had been performed.

	Static		Radial 1		Radial 2		Radial 3	
Group	Patient	Control	Patient	Control	Patient	Control	Patient	Control
N (%)	18 (64)	26 (84)	27 (96)	26 (84)	25 (89)	29 (94)	25 (89)	29 (94)

Table 6.1 Number of participants remaining after removal of bad datasets.

6.5.2 Visual gamma

To identify any differences between the 3 repetitions of the radial block a two-way repeated measures ANOVA was performed. This showed there was no main effect of time on transient gamma for the moving radial blocks for both frequency ($F(2,49) = 0.708$, $p=0.495$) and amplitude ($F(2,49) = 1.911$, $p=0.153$). There was no interaction between time and group for frequency ($F=0.104$, $p=0.902$) and amplitude ($F=2.552$, $p=0.083$). Between subjects analysis showed a main effect of group on frequency ($F(1,49) = 32.570$, $p=<.001$) and amplitude ($F(1,49) = 4.117$, $p=0.046$). The difference in frequency and amplitude between groups for the static block were not significantly different ($t(1,42) = 1.595$, $p = 0.118$; $t(1,42) = -0.077$, $p = 0.939$ respectively). Please see Figure 4A-B.

Controls demonstrated higher sustained gamma frequencies and amplitudes than patients consistently across all runs of the radial task and the static task. A two-way repeated measures ANOVA showed there was no main effect of time on sustained gamma for the moving radial blocks for both frequency ($F(2,49) =$

2.254, p=0.110) and amplitude (F(2,49) = 1.124, p= 0.301). There was also no interaction between time and group for frequency (F=0.956, p=0.388) and amplitude (F=0.476, p=0.623). Between subjects results showed a main effect of group on frequency (F(1,49) = 7.091, p = 0.010) but not amplitude (F(1,49) = 3.081, p = 0.085). The difference in frequency and amplitude between groups for the static block were not significantly different (t(1,42) = 0.839, p = 0.406; t(1,42) = 0.479, p = 0.635 respectively). Please see Figure 4C-D. Appendix 2 shows the means and standard errors for each group and the sample as a whole for each gamma measure and run.

6.5.3 Comparison of static and moving radial gratings

The radial task (Figure 6.2B-D) produced much clearer sustained gamma band spectra within both patients and controls compared to the static task (Figure 6.2A).

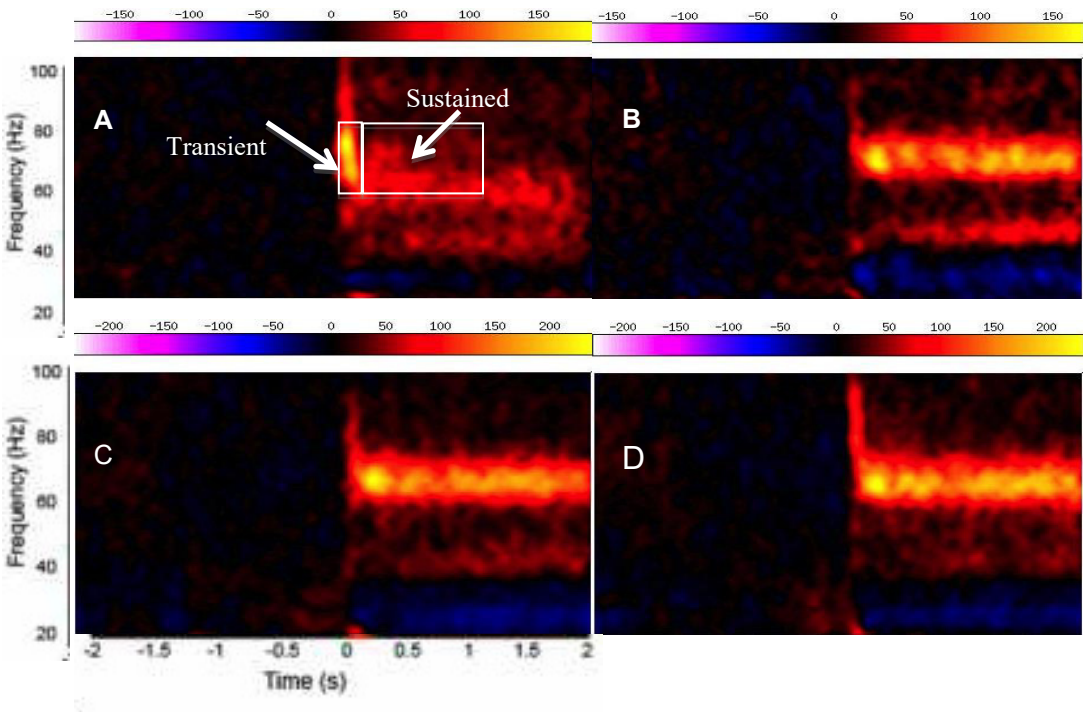


Figure 6.2 Example spectra. A) Static task. B-D) Repeated radial blocks showing clearer sustained gamma.

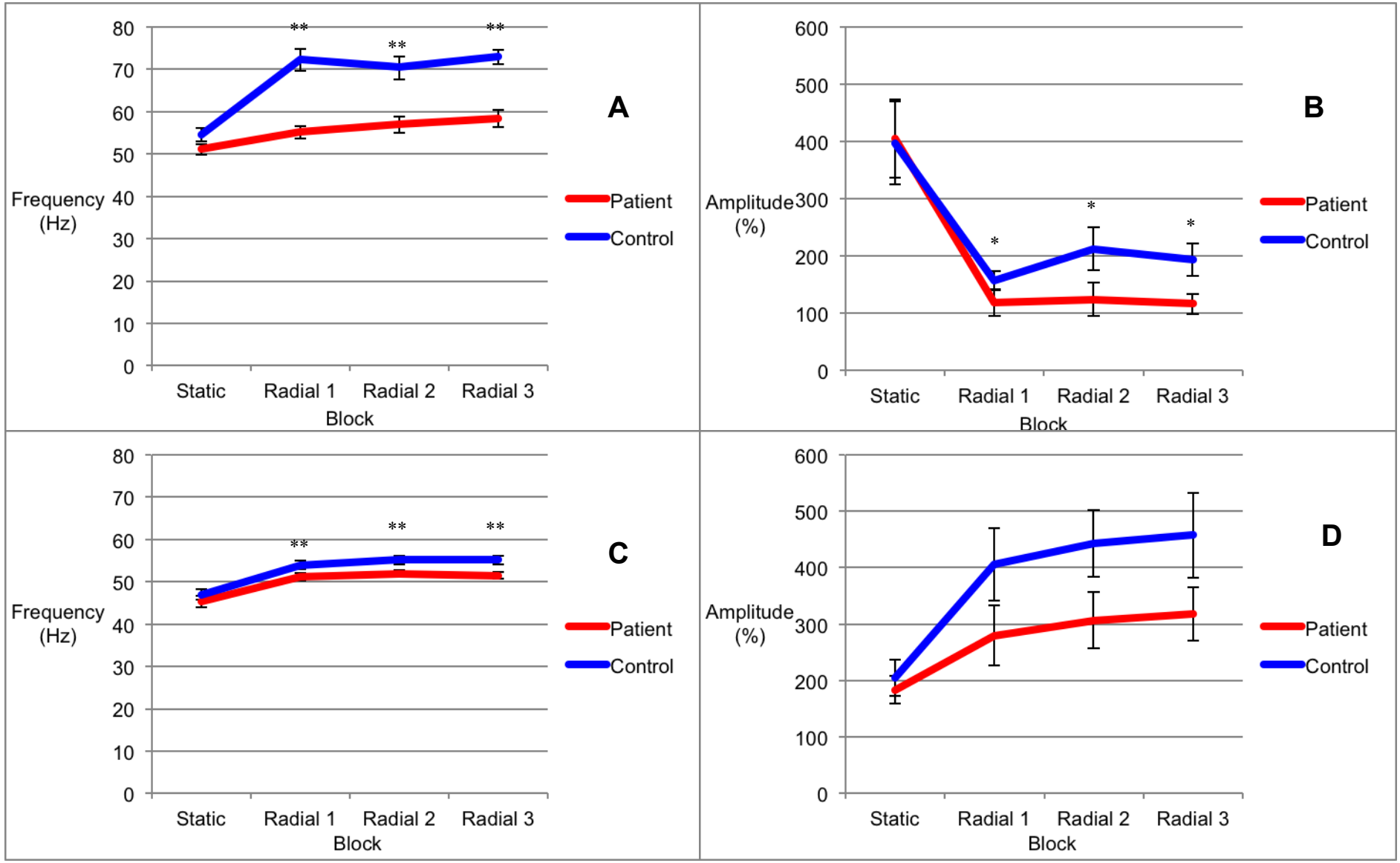
As no differences were found between blocks for the radial stimuli, the results reported for the following analyses will be based on averaging measures across all three radial blocks.

Using a paired t-test split by group, a significant increase was found for transient frequency for the moving task compared to the static within the control group ($t(25) = -7.203$, $p=<.001$) and patients ($t(16) = -4.682$, $p=<.001$). There was also a significant reduction in transient amplitude for the moving task compared to the static task for controls ($t(25) = 2.817$, $p=0.009$) and patients ($t(16) = 4.773$, $p=<.001$). Significant increases in sustained gamma frequency and amplitude were found for the moving task compared to the static within the control group, ($t(25) = -6.394$, $p=<.001$; $t(25) = -5.416$, $p= <.001$ respectively) and the patient group ($t(16) = -7.267$, $p=<.001$; $t(16) = -4.607$, $p = <.001$ respectively). Please see Figure 6.3.

We investigated how parameters estimated in the static experiment (amplitude/frequency) correlated with those extracted from the average moving radial experiment, using Pearson correlation. Transient gamma amplitude was not highly correlated within the whole group or in the control group but produced a higher moderate correlation in the schizophrenia cases - although this did not reach significance. Sustained amplitude showed a significant positive correlation within the sample as a whole and within the patient and control groups separately. Transient frequency moderately correlated for both stimuli with a negative correlation reported within the patient group and a much weaker positive correlation reported within the control group. Sustained frequency

significantly correlated in the sample as whole and in the patient group with a weaker result within the control group (please see Table 6.2 and Figure 6.4).

To summarise, inter-experiment correlations were highest for the sustained measures and were the strongest for measures of gamma amplitude. Gamma frequency demonstrated a weaker correlation, but this perhaps is to be expected as previous studies have shown the change in gamma frequency between static and moving paradigms is dependent on the peak response frequency for the static condition. As no differences were found between the radial blocks for either group, this also suggests a high level of repeatability for this stimulus.



[i]**Figure 6.3**. Transient and sustained gamma frequency (A and C) and Amplitude (B and D) across all 4 experimental runs for Patients. Error bars show +/- 1 Standard Error on the Mean. * = $p<0.05$ ** = $p<0.01$

Transient All	Radial average amplitude	Radial average frequency	Static amplitude	Static frequency
Radial average amplitude		0.331	0.257	0.355
Radial average frequency			-0.108	0.213
Static Amplitude				0.066
Static Frequency				
Control	Radial average amplitude	Radial average frequency	Static amplitude	Static frequency
Radial average amplitude		0.159	0.160	0.429
Radial average frequency			-0.160	0.193
Static Amplitude				0.054
Static Frequency				
Patient	Radial average amplitude	Radial average frequency	Static amplitude	Static frequency
Radial average amplitude		0.278	**0.513***	0.069
Radial average frequency			0.137	-0.385
Static Amplitude				0.120
Static Frequency				

Sustained All	Radial average amplitude	Radial average frequency	Static amplitude	Static frequency
Radial average amplitude		0.308	**0.723***	**0.492***
Radial average frequency			0.246	**0.543***
Static Amplitude				0.298
Static Frequency				
Control	Radial average amplitude	Radial average frequency	Static amplitude	Static frequency
Radial average amplitude		0.264	**0.762***	**0.589***
Radial average frequency			0.288	0.451
Static Amplitude				0.386
Static Frequency				
Patient	Radial average amplitude	Radial average frequency	Static amplitude	Static frequency
Radial average amplitude		0.225	**0.597***	0.271
Radial average frequency			0.92	**0.705***
Static Amplitude				0.082
Static Frequency				

Table 6.2 Pearson correlation coefficients for transient and sustained gamma. * = $p<0.05$ (uncorrected)

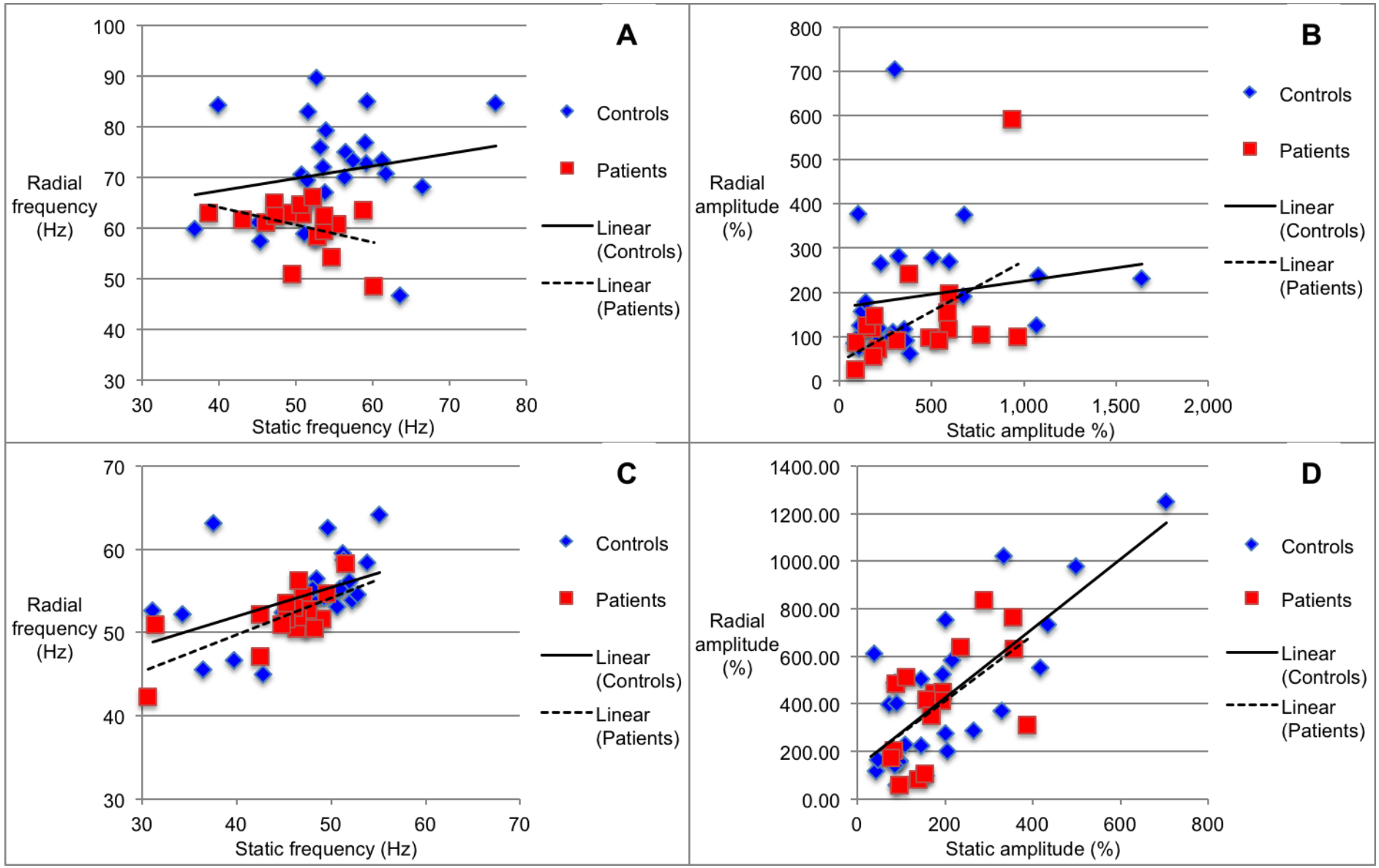


Figure 6.4 Correlations of transient and sustained gamma frequency (A and C) and amplitude (B and D) for static and radial stimuli. Line show line of best fit

6.5.4 Symptom measures

Pearson correlation analyses were performed on both stimuli for both transient and sustained amplitude and each of the symptom measures; BDI-II, Altman Mania Scale, SAPS and SANS. As scores for the SAPS and SANS were zero for each participant in the control group, no analysis was performed on these measures for this group. There was a significant correlation between SAPS score and sustained radial frequency within the patient group (r=-0.510, p=0.028 (p corrected for multiple testing); see Figure 6.5). For all other correlations see Appendix 3.

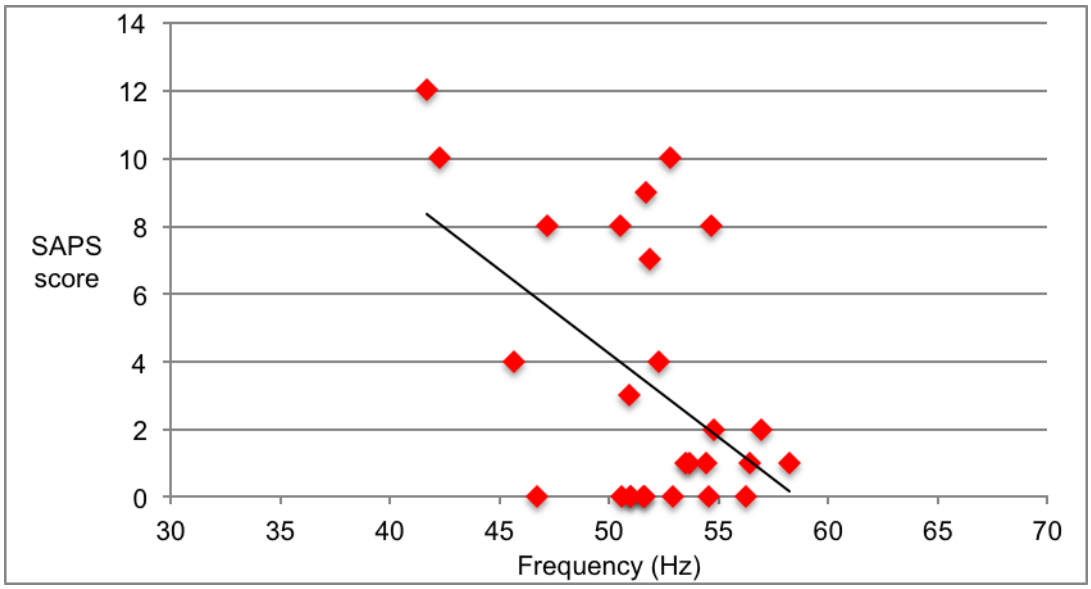


Figure 6.5 Scatter-plot of correlation between sustained radial frequency and SAPS score within the patient group. Line shows line of best fit.

6.5.5 Gamma and GABA

Transient gamma and occipital GABA levels correlated within both groups with moderate positive correlations reported for the control group for radial amplitude and static frequency and a moderate negative correlation reported for patients for radial frequency (see Table 6.3). Sustained gamma and occipital GABA levels correlated more within the patient group than the control group with moderate positive correlations reported with sustained static amplitude and

radial frequency and a moderate negative correlation with sustained static frequency (see Table 6.4). However, none of these correlations were significant after correction for multiple comparisons (corrected p-value 0.0125).

Transient		Radial amplitude	Radial frequency	Static amplitude	Static frequency
Occipital GABA	Controls	0.385	0.285	0.203	0.353
	Patients	-0.231	-0.325	0.111	-0.104

Table 6.3 Pearson correlation coefficients for transient gamma

Sustained		Radial amplitude	Radial frequency	Static amplitude	Static frequency
Occipital GABA	Controls	0.167	0.072	-0.014	0.081
	Patients	0.130	0.344	0.447	-0.325

Table 6.4 Pearson correlation coefficients for sustained gamma

6.5.6 Medication effects

Olanzapine equivalents did not significantly correlate with either task for both sustained and transient frequency and amplitude in the patient group with R^2 values ranging from 0.0001-0.1823.

6.5.7 Structural measures

Freesurfer analysis was performed on the anatomical MRI of each participant to derive measures of cortical surface area, thickness and volume for left and right hemisphere area V1. Table 6.5 shows the mean values for each of the measures for the sample as a whole and for each group.

	Left V1 thickness (mm)	Right V1 thickness (mm)	Left V1 area (mm^2)	Right V1 area (mm^2)	Left V1 volume (mm^3)	Right V1 volume (mm^3)
All	1.91	1.85	2347.76	2216.82	4886.96	4425.76
Patient	1.89	1.84	2376.33	2256.48	4944.04	4484.55
Control	1.93	1.86	2320.21	2178.57	4831.93	4369.07

Table 6.5 Mean values of cortical thickness, area and volume

When correlating the left and right hemispheres each measure was highly correlated across the entire sample and within each group. See Table 6.6 and Figure 6.6.

		Thickness	Area	Volume
All	**r =**	0.749	0.794	0.764
	p =	<.001	<.001	<.001
Patient	**r =**	0.795	0.718	0.874
	p =	<.001	<.001	<.001
Control	**r =**	0.656	0.831	0.602
	p =	<.001	<.001	0.001

Table 6.6 Correlation coefficients and p-vales for left and right hemisphere measures

Finally, Independent sample t-tests showed no between group differences in any of these measures and no significant correlations between the structural measures and transient and sustained frequency or amplitude were found for either task. R^2 values ranged from 0.00003-0.19713.

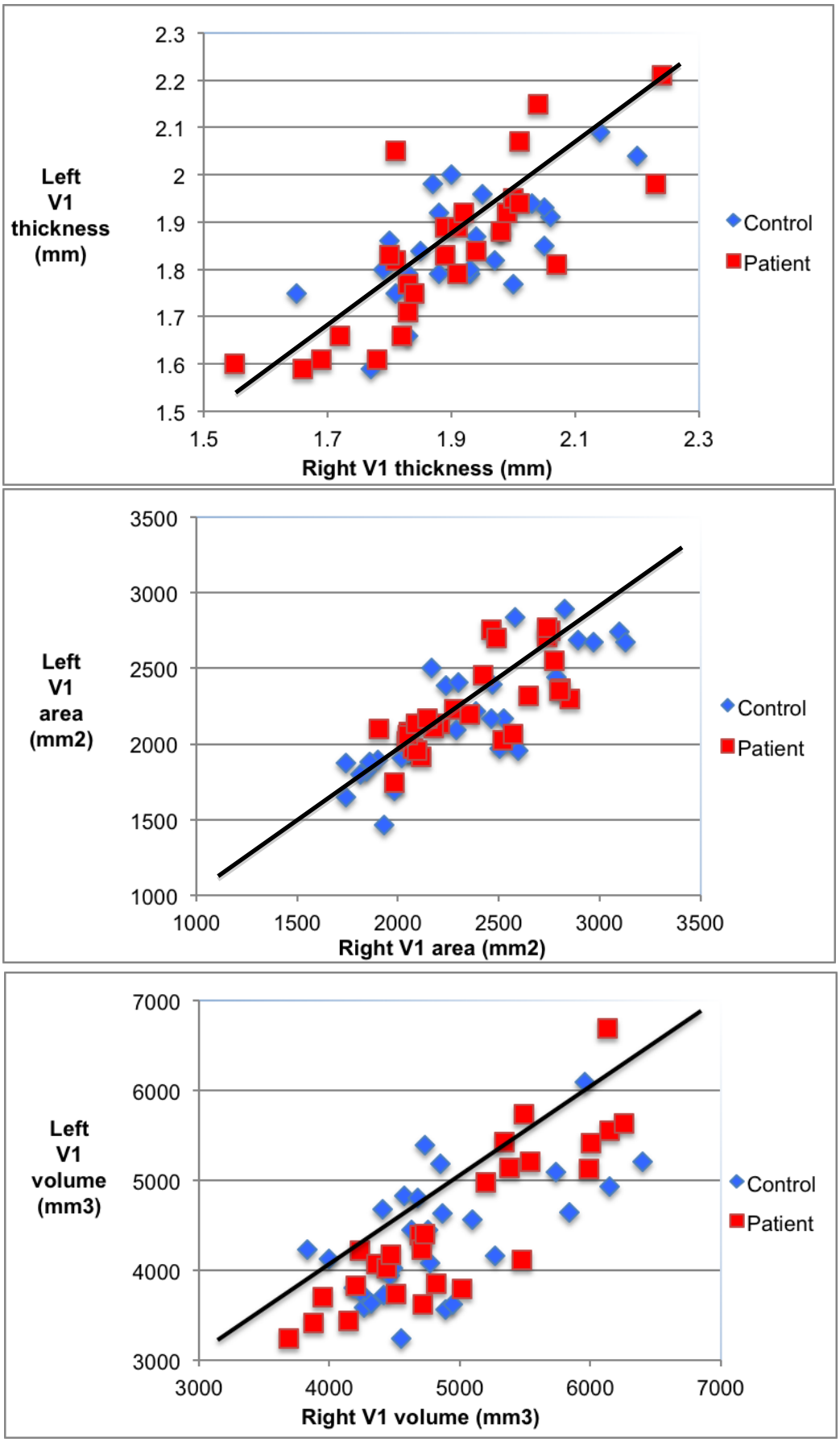


Figure 6.6 Scatter plots showing relationship between left and right V1 thickness (A), area (B) and volume (C). Line shows x=y.

6.6 Discussion

These results reveal a consistent impairment in induced visual gamma frequency and amplitude in patients with schizophrenia compared to age and gender matched healthy controls, irrespective of task. Whilst the differences in amplitude appear more striking, the differences in frequency were also highly significant, suggesting both measures will be key for investigation in the future.

The lack of a difference between groups when using a static task is interesting, as Chen and colleagues also reported no between group differences in rest gamma amplitude (Chen et al., 2014). The difference only occurred when participants were performing a working memory cognitive task. As stated in Chapter 6.2, the moving radial stimulus requires more attention than the static, thus, the necessary coordination of neural activity needed for strong cognitive performance, thought to be reduced in patients, could account for this result. Furthermore, Chen et al also found an associated increase in gamma occurs when the cognitive demand of the task increases. Our findings somewhat support this theory as gamma amplitude and frequency did increase for both patients and controls when performing the more demanding task. However, the difference between the patients and controls only existed when performing the more demanding task suggesting whilst increased cognitive demand may increase gamma activity it may also exacerbate the difference between patients and controls leading to this pattern of results.

Synchrony among large populations of neurons is thought to be essential for normal oscillatory activity (Engel et al., 2001; Uhlhaas and Singer, 2010), particularly in relation to fast-spiking parvalbumin+ interneurons. However, reductions in gamma amplitude have also been linked to excitatory mechanisms and their effects on inhibitory mechanisms. Work with AMPA receptor knock out mice showed the weakening of excitatory connections to fast spiking interneurons reduced the amplitude of gamma oscillations (Fuchs et al., 2007). In addition to these findings, Wiedholz, Owens, Horton, Feyder, Karlsson, Hefner et al, 2008) showed how AMPA knockout mice exhibited symptoms and behaviours characteristic of schizophrenia. As reductions in AMPA lead to reductions in excitatory connections to inhibitory neurons, the reduction in inhibition found in schizophrenia could be excitatory in nature. This hypothesis of a reduction in excitation could account for our reductions in gamma activity in the patient group and the lack of association with occipital GABA levels. Future work should relate gamma activity to excitatory glutamate levels as well as GABA levels to test this theory.

Reproducibility of the moving radial task was tested through three repetitions of the testing block. There were no significant differences between any of the three blocks administered in both the patient and control groups, suggesting high inter-block repeatability and good task compliance across groups. This high inter-block repeatability demonstrated in patients suggests that their reduced gamma frequency and amplitude is not simply an indication of lack of task understanding

given this would lead to random responses which would be inconsistent between blocks.

Fatigue may be an issue when the radial task is administered for as long as it was in this study. When reviewing the raw data some patients' gamma activity dips slightly whilst performing the final radial block. Whilst it could indicate a general lack of performance this would not appear to be the case as correct response rate during this block was comparable between the two groups. Therefore the amount of time participants are asked to complete for this type of task should be considered in future research.

As expected from previous research in healthy controls (Hoogenboom et al., 2006; Swettenham et al., 2009) the moving radial task demonstrated both higher sustained gamma amplitude and frequency for both groups compared to the static task. However, it unexpectedly showed lower transient onset gamma amplitude. This could be due to the reduced onset transient of the stimulus as the static stimuli present at full contrast, within one frame, whereas the radial stimuli ramp up in contrast during initial presentation.

The increase in gamma frequency from static to moving replicates previous findings showing moving and attentionally engaging tasks increase gamma frequency and amplitude (Hoogenboom et al, 2006; Sweetenham et al., 2009;). However, these results also demonstrated possible ceiling effects as most participants' frequencies were being

brought up to approximately 65-70Hz, with a reduction in variation within the groups during the moving task, irrespective of their frequencies during the static task. Whilst the reduction in variation is small and only within the first two radial blocks, this observation needs to be factored in to the design of future studies using this task.

We found no evidence that structural measures of V1 cortical surface area, thickness or volume correlated with any of the imaging measures. This is important because it suggests the between group differences are more likely to be due to abnormalities in inhibitory/excitatory circuitry within the patient group and not just the result of a reduction in gray matter in this group.

We also found no significant correlations between GABA concentration and any of our MEG measures. This is perhaps unsurprising as recent results have been very inconsistent in controls as well as patients. A recent study has failed to replicate the original finding by Edden and collegues in healthy controls with a much larger sample (Cousijn, et al, 2014). However, this relationship has been found when pooling control and patient data for both gamma frequency and amplitude (Chen et al, 2014). However, as this result used a combined sample it should be interpreted with caution due to the previously reported differences in oscillatory activity between schizophrenia cases and controls in this study.

This and previous experiments may not have, therefore, focused on the right measure of GABAergic functioning. Frankle, Cho, Prasad, Mason,

Paris, Himes et al (2015) measured radiolabelled flumazenil, a benzodiazepine-specific PET radiotracer, before and after administration of tiagabine, a GABA membrane transporter (GAT1) blocker in patients with schizophrenia and healthy controls. Blocking GAT1 increases extracellular GABA, which was measured as an increase in flumazenil tissue volume. Flumazenil binding was significantly increased across all brain regions in the healthy controls but not in the schizophrenia group and was positively associated with gamma power in the healthy control group but not the schizophrenia group. Future studies could therefore investigate individual variability in Flumazanil binding within the visual cortex and relate this to individual measures of visual gamma amplitude/frequency in both patient and control groups.

The findings presented here contradict a recent study by Brealy, Shaw, Richardson, Singh, Muthukumaraswamy and Keedwell (2014) who found increases in sustained gamma amplitude in a schizoaffective bipolar disorder sample. Findings in this experiment show quite a marked reduction in gamma amplitude in the schizophrenia group, although the findings for sustained amplitude did not reach significance. The opposite direction of results between these two studies could result from the fact that the patient samples were from distinct diagnostic categories and it may be that features specific to the two disorders are associated with differing GABA profiles. An alternative explanation is that differing medication profiles between these patient groups are responsible for the differences, for example mood stabilisers, much more commonly prescribed in schizoaffective disorders could have contributed ot the

results – the effects of these drugs on MRS GABA have not been studied and are not reported in the Brealy study. These two different disorders should not be combined into one single sample for case/control studies as this could prove to be a source of noise in their results.

6.7 Conclusion

The main finding from this experiment demonstrated patients having clear reductions in visually induced gamma amplitude and frequency when compared to healthy controls when using a radial task but not when using a static task. This is an important finding as it further highlights the possibility of disturbed inhibitory/excitatory mechanisms in this patient group.

A moving radial task elicited stronger visually induced gamma frequency and amplitude in both patients with a diagnosis of schizophrenia and controls. Additional analyses showed structural measures and GABA levels did not drive these between group differences and did not explain a significant amount of individual variability.

Chapter 7 - Linking visual behavioural performance to non-invasive neuroimaging measures of GABAergic function in Schizophrenia and healthy controls

7.1 Rationale

In earlier chapters we have investigated the reliability and parameter dependency of psychophysical tasks that probe inhibition across space (the tilt illusion) and local inhibitory control (orientation discrimination). Animal and human evidence have linked these tasks to GABAergic processes, including studies of healthy human controls and patients with schizophrenia. Similarly we have shown how MEG and MRS can non-invasively probe parameters, such as gamma oscillation amplitude/frequency, that are also thought to be dependent on GABAergic inhibition (as well as glutamatergic excitation). Here, we present the first study to look at measuring in-vivo GABA levels, gamma band measures and psychophysics measures, in the same population of patients and controls. This experiment hopes to investigate two issues; firstly whether the patients demonstrate poorer performance in the psychophysical tasks and secondly whether there is any relationship between the imaging and psychophysics measures. Should a relationship exist, the intention would be to develop the psychophysics into possible new biomarkers for patients with schizophrenia that are grounded in our knowledge of the underlying neurophysiology. These could then be easily administered in a clinical setting as a proxy for the imaging measures.

7.2 Background

7.2.1 Orientation tuning and neuronal selectivity

GABAergic inhibition has been shown to modulate neuronal selectivity in different sensory modalities including auditory (Müller and Scheich, 1987; Wang, Caspary and Salvi, 2000), somatosensory (Dykes, Landry, Metherate and Hicks, 1984; Puts, Edden, Evans, McGlone and McGonigle, 2011) and visual (Alitto and Dan, 2010). The tuning of visual cortical neurons to different orientations is one of the best understood examples of visual selectivity since it was first described by Hubel and Wiesel (1959), with GABAergic inhibition heavily implicated in the tuning of visual neurons to stimulus orientation. Li, et al (2008) showed how administration of GABA agonists to the visual cortex results in sharpening of orientation tuning curves whereas administration of GABA antagonists resulted in broader orientation tuning curves (Sillito et al., 1980; Wolf, Hicks and Albus, 1986; Katzner et al., 2011).

Supporting evidence for the role of GABAergic inhibition in orientation tuning has come from both animal and human research, with Ringach, Hawken & Shapley (2003) showing that, in macaque monkeys, orientation tuning in V1 is a process driven by rapid excitation and equally rapid inhibition, possibly GABAergic. As discussed in the General introduction chapter, in human research Edden et al (2009) showed a negative correlation between GABA levels within the visual cortex and orientation discrimination (OD) thresholds demonstrating that increased GABA levels resulted in lower thresholds and greater success in completing this task. A recent study (Dickinson et al., 2015) investigated the relationship between

autistic traits, OD thresholds and peak gamma frequency in a healthy sample. In line with previous findings that higher peak gamma frequency and lower discrimination thresholds are related to levels of GABAergic inhibition (as evidenced also by Edden et al, 2009), they found significant negative correlations between thresholds and peak gamma frequency and autistic traits. This lends further support to inhibition playing a major role in orientation selectivity and the ability to perform well on an OD task.

These findings have also been related to psychiatric disorders consistent with findings of a reduction in occipital (visual cortex) GABA levels in patients with schizophrenia, patients have been found to have significantly wider orientation tuning curves compared to healthy controls (Rokem, et al 2011). However, there has been very little research conducted with patients using the OD task used previously in this thesis to ascertain whether a deficit occurs and whether an oblique effect, as defined in the General introduction chapter, is present within this patient group.

7.2.2 Tilt illusion/surround suppression and context modulation

As discussed in the General Introduction, the Tilt Illusion (TI) is an example of a perceptual phenomenon where the introduction of an additional stimulus modulates the properties of a central stimulus by biasing the orientation of the simultaneously presented central stimulus (Gibson, 1937). This is also known as contextual modulation. Like Chapter 4, the experiments in this chapter will focus on the direct effect. This refers to an oriented surround producing a repulsive effect on the perceived orientation of a central stimulus.

Contextual modulation has previously been explained as interactions between neighbouring neurons that mutually influence each other, for example via lateral inhibition of neurons or sensory gain control (Clifford, 2014). Sensory gain control is an extension of lateral inhibition referring to a process that allows, in this instance, the visual system to adapt its responses to stimuli to take into account spatial and temporal context (Butler et al., 2008). The level of gain control is proposed to be weaker in patients with schizophrenia than in controls (Butler et al, 2008; Phillips and Silverstein, 2013) leading to a reduction in contextual modulation as demonstrated by Dakin et al (2005); Serrano-Pedraza et al., (2014) who found reduced contextual modulation in a patient group when measuring perceived contrast and by Yoon et al (2009) when measuring orientation selectivity.

Imaging studies have supported the idea of reduced levels of contextual modulation in schizophrenia with recent fMRI studies providing strong neurophysiological evidence for this theory having shown abnormally weak orientation specific contextual modulation in the visual cortex of patients with schizophrenia (Seymour et al., 2013). From this it would be expected that patients would have weaker direct effects when using the TI task. However, as discussed in the General introduction, when investigating the direct effect in patients the results are mixed. Both Yang et al., (2013) and Tibber et al., (2013) found no difference between patients and controls in the magnitude of the direct effect. In addition, Yang found that larger direct effects within the patient group were

associated with higher symptom severity whereas Tibber and colleagues found the opposite when relating to symptom severity.

These results are contrary to what intuitively would be expected given the results of Butler et al, 2008; Philips and Silverstein, 2013 and Dakin et al (2005), and also Gelbtuch et al (1986) who positively correlated GABA levels (using Lorazepam, a benzodiazepine that potentiates activity at GABAa receptors) with the size of the direct effect using the tilt illusion in healthy controls. Given this demonstrated pharmacological effect, we have here chosen the tilt illusion to be our probe of contextual modulation effects that we can assume are related to GABAergic processes. Given the previous findings of a reduction in GABA levels in patients with schizophrenia it would be expected that this group would not be so affected by the introduction of a surround when compared to healthy controls thereby resulting in weaker direct effects.

There is limited research into the (TI) specifically and schizophrenia. Therefore more research is needed using the (TI) within this patient group to investigate how contextual modulation relates to this disorder and how this could be mediated by neurophysiological factors.

7.3 Aims

The aim of this experiment was to investigate the differences in performance on the OD and TI tasks between patients with a diagnosis of schizophrenia and healthy controls. The results from these tasks would then be related to imaging measures, specifically occipital GABA levels

and gamma band oscillations, and symptom measures. It was expected that patients would perform worse on the OD task resulting in higher discrimination thresholds, and exhibit smaller direct effects compared to controls. In addition, thresholds are expected to positively correlate with symptom measures whereas the sizes of participants' direct effects are expected to negatively correlate.

7.4 Methods

7.4.1 Participant sample and recruitment

Participants used in the control and patient groups for the psychophysics and imaging data are identical to those recruited for Chapters 5 and 6.

7.4.2 Psychophysics methods

Orientation discrimination methods used for this experiment were identical to those described in Chapter 3 with the exception that the conditions administered in this experiment were 250ms vertical, 10ms vertical, 250ms oblique and 10ms oblique. Tilt Illusion methods used in this chapter are identical to those described in Chapter 4.

7.4.3 Imaging methods

Magnetoencephalography (MEG) methods are identical to those described in Chapter 6. Both visual tasks described were used for the purpose of this experiment. Magnetic resonance imaging (MRI) and spectroscopy (MRS) methods used for this experiment were identical to those described in Chapter 5. Only occipital GABA measures were used for analysis in this experiment.

7.4.4 Behavioural and anti-psychotic medication measures

All behavioural measures and anti-psychotic medication equivalents used for this experiment are identical to those described in Chapter 5.

7.4.5 Correlating psychophysical thresholds with imaging measures

Participants included for further analysis in each of the imaging measures are identical to those identified in Chapter 5 (GABA measures) and Chapter 6 (gamma measures). Pearson correlation coefficients were calculated on the PSE differences calculated from the TI, thresholds from each of the OD blocks and all imaging outcome measures.

7.5 Results

OD and TI results were excluded from further analysis if they were +/- 2 standard deviations from the mean of that condition in line with previous research (Edden et al, 2009). See Table 7.1 for remaining participant numbers for each task and condition.

	N (%) controls remaining	**N (%) patients remaining**
PSE	27 (87)	24 (86)
250ms vertical	28 (90)	25 (89)
10ms vertical	28 (90)	26 (93)
250ms oblique	29 (93)	26 (93)
10ms oblique	29 (93)	26 (93)

Table 7.1 Participants remaining after exclusion of outliers

7.5.1 Psychophysics: The tilt illusion

As in Chapter 4, the magnitude of the tilt illusion effect in each person was quantified using the PSE (point of subjective equality). A PSE of zero

represents no tilt illusion. The size of the direct effect was quantified by subtracting the baseline no surround condition PSEs from the surround PSEs. All PSE differences calculated were positive, indicating a repulsive effect. Patients exhibited higher PSE differences (stronger direct effects) compared to controls (see Figure 7.1). However univariate ANOVA analysis showed this difference was not significant F(49) = 1.616, p=0.210).

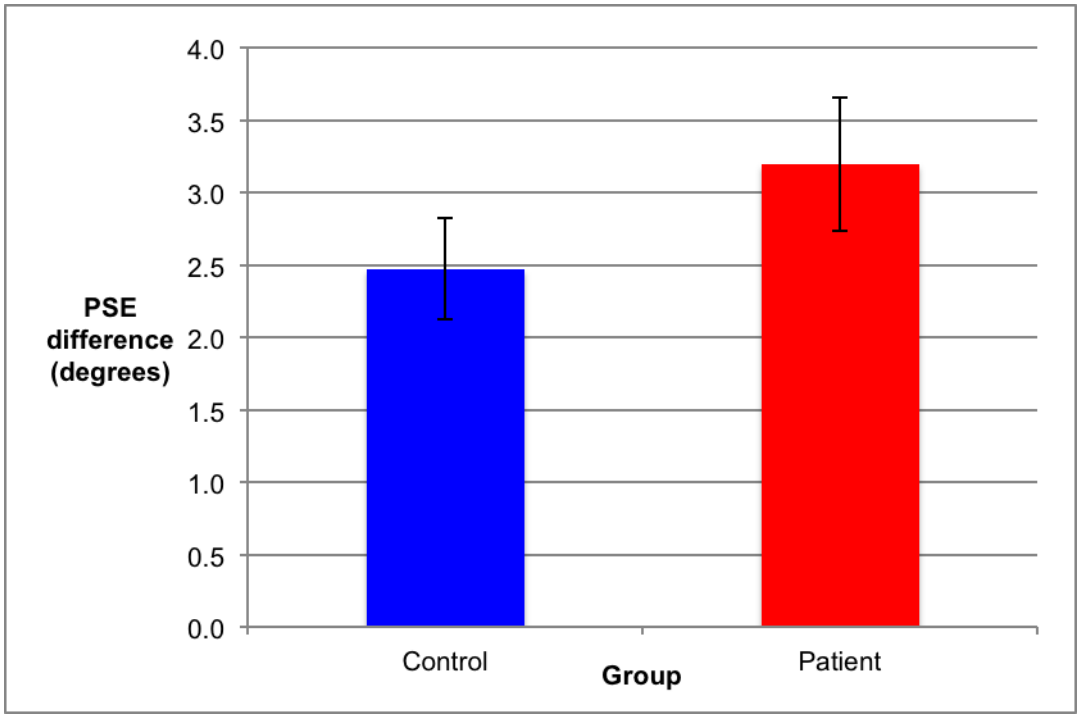


Figure 7.1 Mean PSE differences for both control and patient groups. Error bars show +/- one standard error of the mean

7.5.2 Psychophysics: Orientation discrimination

Figure 7.2 shows the mean thresholds calculated for each duration (250ms or 10ms) and each orientation (vertical or oblique). It also shows the difference between the patient and control groups. Univariate ANOVA analyses showed highly significant between-group differences in each of the four conditions of the orientation discrimination (OD) task; 250ms vertical (F(51) = 9.957, p=.003), 10ms vertical (F(53) = 15.034, p=<.001),

250ms oblique ($F(52) = 25.774$, $p=<.001$ and 10ms oblique ($F(52) = 19.152$, $p=<.001$).

A repeated measured ANOVA demonstrated a significant oblique effect when using a stimulus duration of 250ms in controls ($F(26) =36.964$, $P=<.001$) and in patients ($F(23) = 35.279$, $P=<.001$). The oblique effect was also present when stimulus was presented for 10ms in controls ($F(26) = 51.282$, $P=<.001$) and in patients ($F(25) = 32.551$, $P=<.001$). There was a significant difference in the size of the oblique effect between groups with patients having a larger oblique effect for 250ms ($F(49) = 20.392$, $p=0.000$) and 10ms ($F(49) = 20.319$, $p=0.001$). However, expressed as proportions, the oblique effects were similar in each group, with the vertical to oblique increase for the 250ms and 10ms conditions being respectively 3.574 and 3.294 times larger in the control group and 3.431 and 3.251 times larger in the patient group (see Figures 7.2 and 7.3).

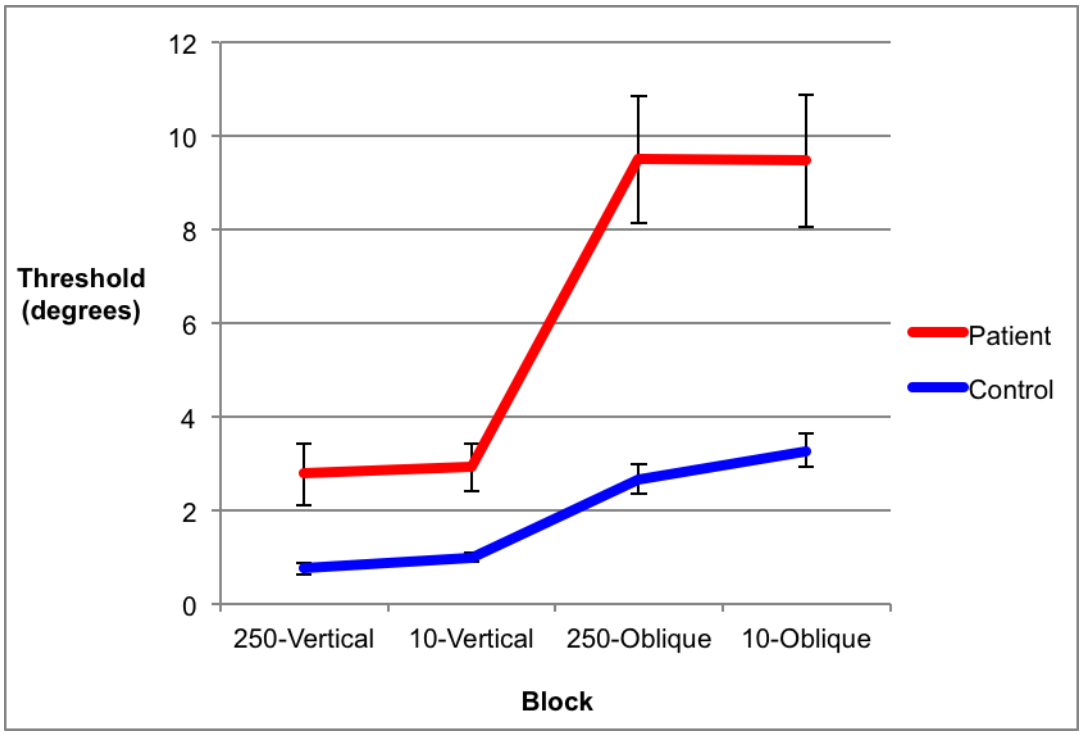


Figure 7.2. Mean orientation discrimination thresholds for both controls and patients. Error bars show +/- standard error of the mean.

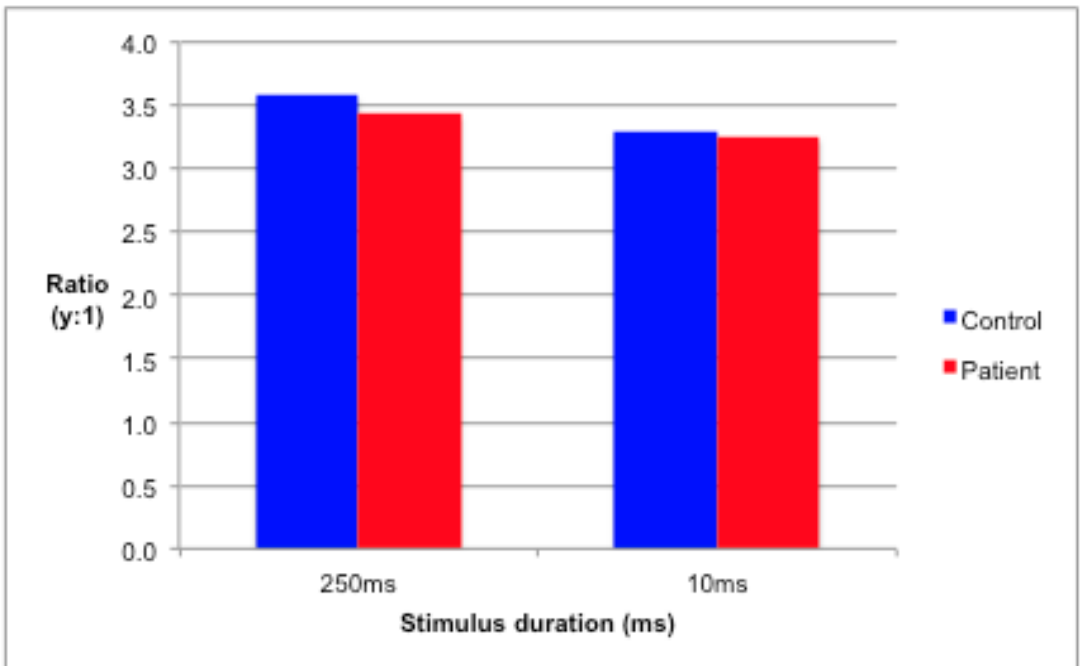


Figure 7.3 Bar chart demonstrating the oblique effect based on different stimulus durations within the control and patient group

An investigation of the dependency of orientation discrimination thresholds on stimulus duration produced a borderline significant effect only for the vertical condition within the control group (F(27) = 4.236, P=0.049) but no significant effect in the control group for the oblique conditions (F(25), = 2.908, p=0.101) or in the patient group for both vertical (F(23) = 0.753, p=0.395) and oblique conditions (F(24) = 0.002, p=0.967), see Figure 7.2.

Pearson correlation tests of between condition repeatability showed that vertical and oblique conditions positively correlated in both groups but were much stronger in the patient group (see Tables 7.2-7.3; key correlations are highlighted in Figure 7.4). At a 250ms duration, the vertical and oblique thresholds were only correlated in the patient group, whereas for 10ms, the vertical and oblique thresholds were highly correlated for both patients and controls. When combining different stimulus durations with different orientations the patient group showed significant moderate correlations whereas in the control group only the 10ms vertical and 250ms oblique conditions correlated. For the means and standard errors of these tasks for both controls and patients, see Appendix 4.

Control group	**250ms vertical**	**10ms vertical**	**250ms oblique**	**10ms oblique**
250ms vertical		**0.476***	0.168	0.306
10ms vertical			**0.495***	**0.623****
250ms oblique				**0.571***
10ms oblique				

Table 7.2 Correlation coefficients within the control group for each orientation discrimination condition. Bold values represent significant r values with a uncorrected p-value <.05. * = p<.05 ** = p<.001

Patient group	250ms vertical	10ms vertical	250ms oblique	10ms oblique
250ms vertical		**0.688****	**0.614****	0.455
10ms vertical			0.460	**0.660****
250ms oblique				**0.652****
10ms oblique				

Table 7.3 Correlation coefficients within the patient group for each orientation discrimination condition. Bold values represent significant r values with a uncorrected p-value <.05 * = p<.05 ** = p<.001

7.5.3 Correlations between Transient Gamma parameters and behavioural measures

In both groups the significant Pearson correlation results were for the 250ms oblique condition of the OD task and static amplitude, with the strength of the correlations being very similar between groups (see Tables 7.4 and 7.5). This result remained significant after correction for multiple comparisons in the control group only. In addition, within the patient group there was a strong correlation between the 250ms vertical condition and radial frequency.

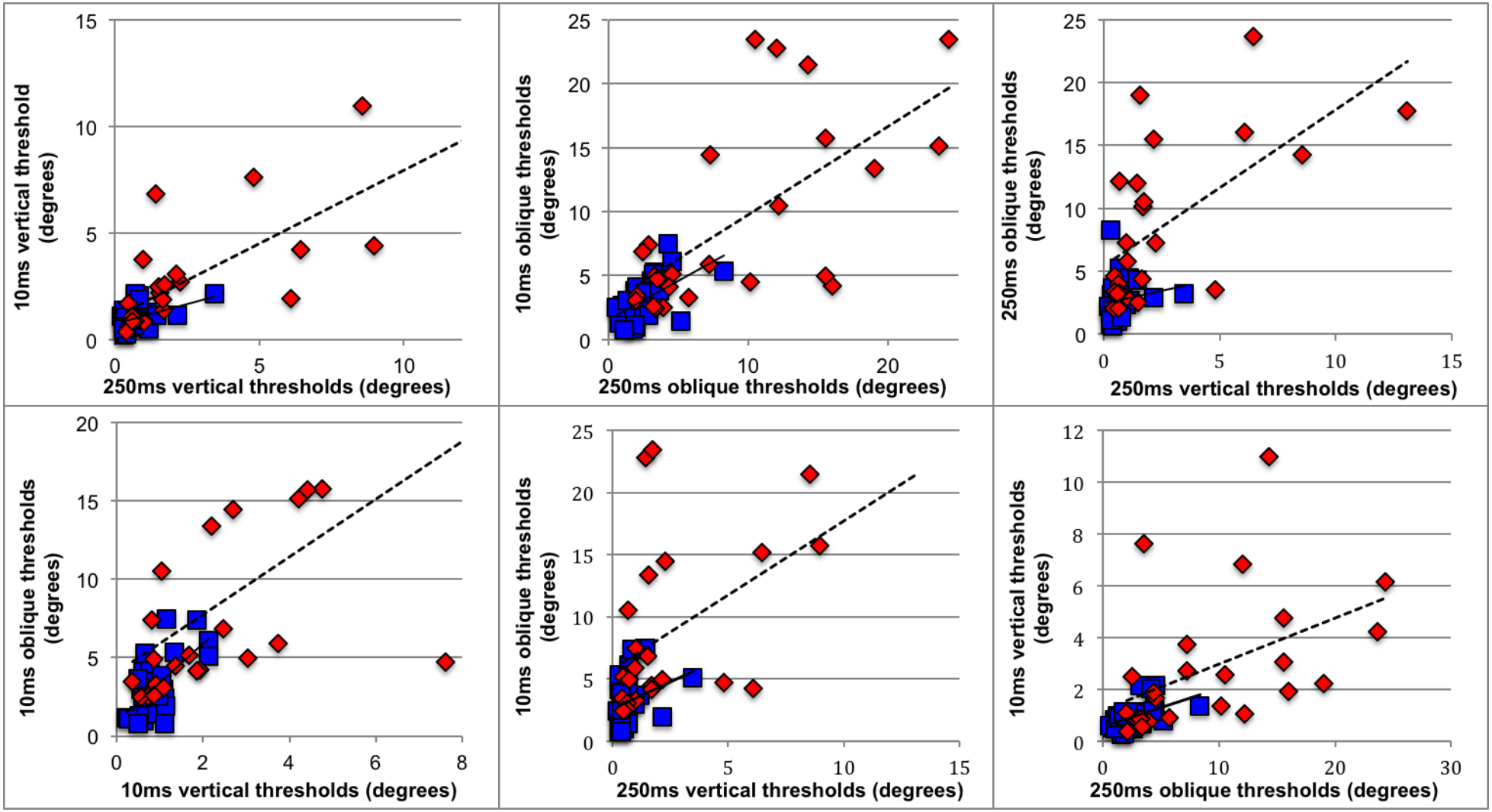


Figure 7.4. Scatter-plots of orientation discrimination blocks in the control (■) and patient (◆) groups. Solid line(▬) shows line of best fit for the control group. Dashed line (------) shows line of best fit for the patient group.

Controls	PSE	250ms vertical	10ms vertical	250ms oblique	10ms oblique
Transient static frequency	-0.382	-0.063	0.090	-0.119	-0.001
Transient radial frequency	-0.369	0.199	-0.173	-0.432	-0.304
Transient static amplitude	-0.177	-0.256	-0.087	**0.535***	0.089
Transient radial amplitude	-0.078	-0.0.37	-0.480	0.105	0.075

Table 7.4 Correlation coefficients within the control group for the transient MEG imaging and psychophysics measures. Bold values represent significant r values with a corrected p-value <.0125 * = p<.0125 ** = p<.001

Patients	PSE	250ms vertical	10ms vertical	250ms oblique	10ms oblique
Transient static frequency	-0.437	-0.283	-0.079	-0.103	-0.082
Transient radial frequency	-0.081	**-0.607***	-0.233	-0.414	-0.817
Transient static amplitude	-0.335	-0.214	-0.329	-0.568	-0.348
Transient radial amplitude	-0.284	-0.225	-0.342	-0.368	-0.183

Table 7.5 Correlation coefficients within the patient group for the transient MEG imaging and psychophysics measures. Bold values represent significant r values with a corrected p-value <.0125 * = p<.0125 ** = p<.001

7.5.4 Sustained gamma

Within the control group all of the oscillatory measures produced weak correlations with the psychophysics measures (see Tables 7.6, 7.7 and Figure 7.5). Within the patient group, sustained radial amplitude produced significant negative correlations with all blocks of the OD task and only a slightly weaker negative correlation with the magnitude of the PSE. The correlations between the 10ms vertical and 250ms oblique conditions and sustained radial amplitude survived correction for multiple comparisons.

Controls	PSE	250ms vertical	10ms vertical	250ms oblique	10ms oblique
Sustained static frequency	-0.183	-0.157	-0.039	0.058	0.090
Sustained radial frequency	-0.008	-0.045	0.172	0.043	0.244
Sustained static amplitude	-0.168	-0.254	-0.006	0.343	0.255
Sustained radial amplitude	-0.078	0.023	0.027	0.256	0.289

Table 7.6 Correlation coefficients within the control group for the sustained MEG imaging and psychophysics measures. Bold values represent significant r values with a corrected p-value <.0125 * = p<.0125 ** = p<.001

Patients	PSE	250ms vertical	10ms vertical	250ms oblique	10ms oblique
Sustained static frequency	-0.410	-0.203	-0.238	-0.393	-0.252
Sustained radial frequency	-0.133	-0.323	0.012	-0.280	-0.190
Sustained static amplitude	0.011	0.000	-0.104	-0.303	-0.045
Sustained radial amplitude	-0.397	-0.410	**-0.486***	**-0.587***	-0.454

Table 7.7 Correlation coefficients within the patient group for the sustained MEG imaging and psychophysics measures. Bold values represent significant r values with a corrected p-value <.0125 * = p<.0125 ** = p<.001

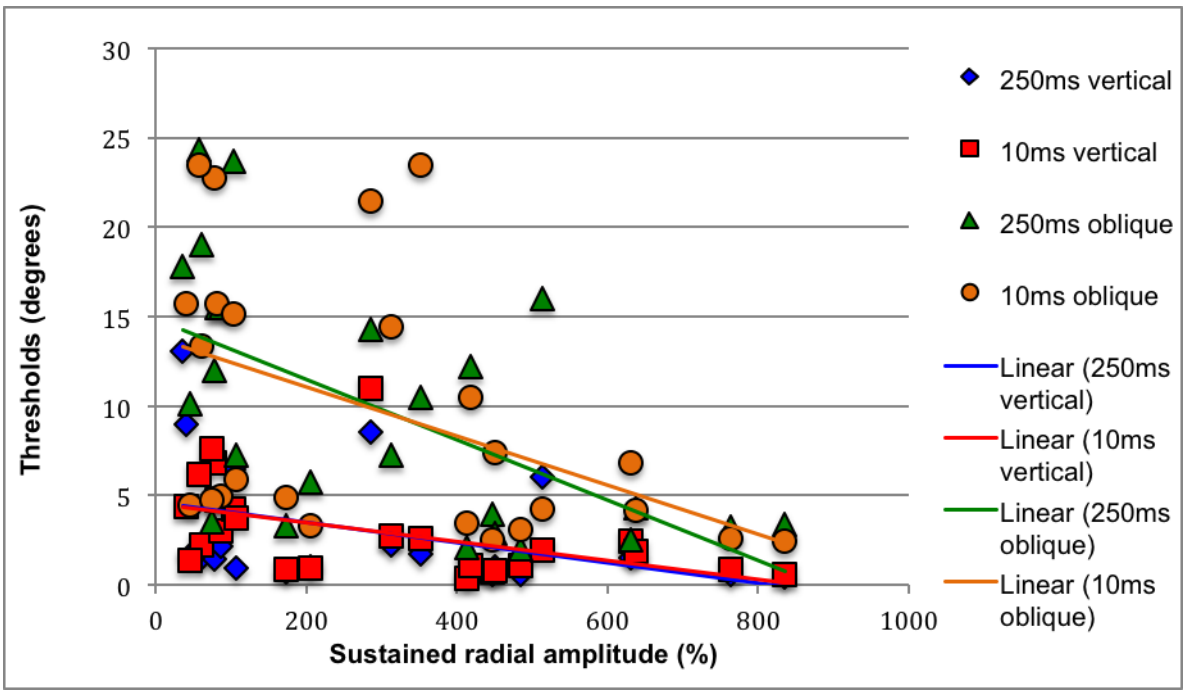


Figure 7.5 Scatter plot showing correlation between orientation discrimination thresholds and sustained radial amplitude in the patient group. Lines show line of best of fit.

7.5.5 Occipital GABA

Table 7.8 shows the only significant correlation found with occipital GABA levels were with the 10ms vertical condition of the OD task; and that this was present only within the control group.

	PSE	250ms vertical	10ms vertical	250ms oblique	10ms oblique
Control Occipital GABA	-0.231	0.268	**-0.417***	0.158	-0.103
Patient Occipital GABA	0.211	0.155	0.070	0.081	0.055

Table 7.8 Correlation coefficients within the control group for the sustained imaging and psychophysics measures. Bold values represent significant r values with a corrected p-value <.01 * = p<.01

7.5.6 Structural effects

Freesurfer analysis was performed on the imaging data to derive measures of cortical thickness, volume and surface area for both hemispheres in the visual cortex. Pearson correlation coefficients were calculated on these structural measures and the psychophysics measures. There were no significant correlations between any of the measures within the control group (all r^2 values <0.084) and the patient group (all r^2 values <0.122).

7.5.7 Medication effects

Within the patient group olanzapine equivalents did not correlate with any of the psychophysics results (all r^2 values < 0.117). For medication effects in relation to the imaging measures please see Chapter 5 (section 5.5) for relationship with GABA levels and Chapter 6 (section 6.5) for gamma measures.

7.5.8 Behavioural measures

The behavioural measures showed low negative correlations between the Beck Depression Inventory II (BDI-II) and OD thresholds within the control group, none of which reached significance. The patients showed the opposite direction of effect, with equally low correlation coefficients within the vertical OD conditions but with moderate positive coefficients for the oblique conditions with the 10ms oblique results reaching significance ($r=0.438$, $p=0.025$). Within the patient group the SAPS scores also moderately correlated with the 10ms oblique condition ($r=0.415$, $p=0.035$). However, these results did not survive correction for multiple comparisons.

7.6 Discussion

The results from this experiment show that patients had a clear impairment in the OD task across all four conditions. This was hypothesised to be the case, as patients with a diagnosis of schizophrenia are known to have broader tuning curves impairing their performance on tasks of this type (Rokem et al., 2011). However these thresholds only weakly correlated with GABA levels. Previous research has strongly implicated reduced inhibition (possibly GABAergic) in the broadening of tuning curves in this patient group (Rokem., 2011). Whilst difference in OD thresholds taken alone does support the idea of a broadening of tuning curves in schizophrenia, the lack of relationship with GABA would suggest this is not GABAergic in nature.

Another key finding was a strong negative correlation reported between sustained radial amplitude and the orientation discrimination thresholds in the patient group. Both of these measures have been strongly related to excitatory

mechanisms with AMPA receptor blockade leading to a reduction in gamma amplitude Fuchs et al., 2007 and the tuning of orientation curves linked to rapid excitation and equally rapid inhibition (Ringach et al., 2003). As the patients demonstrated significantly higher thresholds compared to controls, with these thresholds correlating with gamma amplitude but not occipital MRS GABA levels, it does suggest that these measures could possibly be mediated by excitatory mechanisms and focussing on these would provide a more sensitive measure of inhibitory/excitatory circuit abnormalities and orientation tuning in this patient group.

These results do not replicate those found by Edden et al (2009) in healthy control participants. In this control sample the r value for 250ms oblique thresholds and GABA was small and in the opposite direction of effect; 0.158 compared to -0.65. In contrast we did find a moderately significant negative correlation for the 10ms Vertical condition, which is the same direction as the Edden correlation but for a different stimulus condition. A similar pattern of non-replication was found for gamma frequency where our r value was 0.043 compared to -0.65. The age range in Edden and colleagues study was broad (23.5–42.9) but there sample size was small with final n of 13 participants compared to our 31. Therefore, there is the possibility that the Edden results were produced by chance and that the current study with an increased sample size, reflects truer results.

There were no differences between the 10 and 250ms conditions for either orientation, which was surprising, as an earlier experiment within this thesis had shown a marked increase in thresholds when stimulus duration was decreased,

with these two conditions in particular being different to a highly significant degree (Chapter 3 Figure 3.3). This cannot be due to simple methodological differences, such as order effects or stimuli, because the protocol was identical: in both experiments, the order of blocks administered was counter-balanced with identical stimulus parameters. This suggests other factors lie behind this discrepancy in results such as age of participants. The results from Chapter 3 were obtained from a very narrow sample with the majority being undergraduate students aged between 18-22. The sample in this experiment had a much broader age range of 22-58. Betts et al (2007) demonstrated orientation thresholds increase with age thus it would be expected the results in this study would show increased thresholds compared to those in Chapter 3. This was not the case as the 250ms condition was slightly reduced in the older sample and the 10ms condition mean thresholds were approximately half the size in the older sample compared to the younger. As this is the first study to our knowledge that has investigated orientation thresholds at such small stimulus durations, it is possible that age improves performance at the smaller stimulus durations but has no effect or increases thresholds at the bigger stimulus durations. Replication in an independent sample is needed to verify this result.

For the Tilt illusion, patients exhibited slightly larger direct effects when compared to controls. However, this trend was not significant, consistent with previous research demonstrating no difference between patients and controls using this task (Yang et al, 2008; Tibber et al, 2013). In general therefore, our results do not support the theory that patients with a diagnosis of schizophrenia are not as affected by contextual differences in stimuli when compared to healthy controls.

Due to the nature of their illness, patients with schizophrenia may not fully understand instructions given to them on how to perform the psychophysics tasks and therefore will not complete them reliably. This lack of understanding may then lead to the differences between the groups. However, the strong correlations between the conditions in the OD task suggest this is not the case and that the reduction in thresholds is a real between group difference. In addition the difference between direct effects calculated from the TI was not significant suggesting groups are comparable in their ability to complete this task. Lack of understanding by the patients does not appear to be an issue for this sample.

The reliability of these tasks has also been addressed by these results. The high correlations between OD blocks suggest that there is a high level of individual repeatability across conditions. In addition, as all of the direct effect results were positive, indicating a repulsive effect, in line with previous results (Gelbtuch et al, 1986), it appears that the TI task is valid. Therefore, it appears both tasks produce reliable results in both healthy and schizophrenia populations.

The BDI-II and SAPS positively correlated with the 10ms oblique condition only within the patient group. This replicates previous findings showing a significant relationship between symptom severity and OD thresholds. It is interesting that the measure of positive symptom severity and levels of depression correlate with the most difficult condition of the OD task and not the task as whole. The BDI-II results replicate previous findings as higher levels of depression have

been found to correlate with levels of cognition especially attention, concentration and short-term memory (Trivedi and Greer, 2014), all of which are needed to complete this task but more so for this particular condition. However, results of symptom severity tend to find correlations with negative symptoms and cognition but not positive so these results are opposite to what has previously been found (Harvey, Howanitz, Parella, White, Davidson, Mohs et al, 1998; Keefe, Bilder, Harvey, Davis, Palmer, Gold et al, 2006).

The psychophysics results did not appear to be explained by structural measures or medication within the patient group. This is encouraging and again suggests the results shown are true differences in psychophysics performance between groups and was not mediated by these factors.

For discussion of imaging measures please see the Discussion sections of Chapters 5 and 6.

7.7 Conclusion

Patients exhibited significantly higher orientation discrimination (OD) thresholds compared to controls but were comparable when performing the Tilt illusion. Thresholds calculated from the OD correlated with sustained radial gamma amplitude in the patient group but not with GABA levels. Both of these measures have been linked to excitatory processes, as well as inhibitory, which may explain the lack of relationship with inhibitory GABA levels and point to a

more sensitive measure for linking imaging measures and OD thresholds in the future.

Chapter 8 - General discussion

This thesis utilised psychophysics, MRS and MEG methods in order to gather information relating to cortical inhibition and neural oscillatory activity in patients with schizophrenia and healthy controls.

8.1 Summary of findings

Chapters 3 focused on administering the orientation discrimination (OD) task in healthy controls as possible ceiling effects had been identified in Edden and colleagues experiment. An additional aim was to find a variant on the vertical condition, which produced comparable thresholds to an oblique condition but without the associated loss of data. This experiment demonstrated that the reduction in stimulus duration increased participants' OD thresholds, thus increasing the difficulty of the task in a healthy control population. The smallest and largest stimulus durations correlated very strongly suggesting ceiling effects were not present. In addition, performance at every other stimulus duration correlated suggesting a high level of repeatability within the task itself. However, the hardest vertical condition did not produce comparable thresholds with the oblique condition administered, with them still being ~2.5x higher in the oblique condition.

Chapter 4 investigated the link between the Tilt illusion (TI) and visual gamma whilst additionally piloting the TI in healthy controls. The TI task produced positive results indicating a repulsive direct effect and that the task worked. However, no relationship was found between the size of participants' direct effects and visual transient or sustained gamma amplitude or frequency.

Chapter 5 demonstrated a reduction in occipital GABA levels in patients with a diagnosis of schizophrenia when compared to healthy age and gender matched controls. This result was limited to this cortical location, as GABA measured in the sensory motor region produced no difference between groups. This fits with previous research that shows GABA reductions/increases are different depending on the region in which they are measured (Kegeles et al, 2012) but also level of medication. This experiment found antipsychotic medication had no effect on the occipital GABA result. This finding is supported further by the fact reductions were found in only one voxel whereas if medications were affecting GABA levels across the brain, a reduction should have been found in the sensory motor voxel also.

Chapter 6 compared induced visual gamma activity in patients with a diagnosis of schizophrenia and healthy controls using a static grating stimulus and a moving radial stimulus. Gamma amplitude and frequency were both reduced in patients across all runs of a moving radial stimulus but these differences were much reduced or not evident at all when using a static stimulus. The reduction in gamma activity demonstrated by the patients also replicates previous findings by (Chen et al, 2014). Again, no medication effects were found in this experiment.

In addition, both groups demonstrated a significant increase in sustained frequency and amplitude and transient frequency. These findings compliment previous research in healthy controls showing the use of a moving stimulus induces higher transient and sustained gamma activity (Hoogenboom et al, 2006; Sweetenham et al, 2009) and extends them to include patients. However,

a reduction was seen in transient amplitude when using the moving stimulus compared to the static.

The final chapter of this thesis brought the neuroimaging measures together with the psychophysics measures. It investigated whether there was any difference in performance in both the OD and TI tasks between groups and whether participants' performance correlated with occipital GABA levels and visually induced gamma activity. Results showed patients were significantly poorer at the OD task as demonstrated by the much larger thresholds in each of the conditions administered. Patients also demonstrated larger absolute oblique effects but proportionally they were comparable with the control group. Each condition within this task correlated strongly with sustained gamma amplitude in the patient group but not in the control group.

Patients demonstrated slightly larger direct effects in the TI, however, this difference was not significant when compared with the control group. When relating the size of the direct effects to the imaging no correlation was found between both GABA levels and the majority of gamma measures in either group.

8.2 Implications of main findings and future work

8.2.1 Increase in orientation discrimination due to decrease in stimulus presentation time:

This is the first experiment, that I am aware of, that has identified this link between stimulus duration and discrimination thresholds. The hardest and easiest conditions are highly correlated, strongly suggesting no ceiling effects

were present in using this task, addressing the possibility of this in Edden et al (2009). The fact that each of the vertical conditions were highly correlated suggests participants were not performing at chance level in the harder conditions, indicating all of the conditions administered produced reliable thresholds of orientation discrimination.

However, even though this experiment demonstrated the reduction in stimulus duration produces increases in thresholds, the oblique condition was still producing thresholds ~2.5x higher than the 10ms vertical condition. This suggests that there is no comparable vertical condition and that the oblique condition is still much harder for participants to complete. In itself this is very interesting as the oblique effect is still quite under studied. The fact that thresholds continue to be higher in the oblique condition when compared to harder vertical conditions strongly suggests a different mechanism for this type of orientation discrimination.

8.2.2 No relationship between visual gamma and size of direct effect as measured by the tilt illusion:

This experiment used visual gamma measures as a proxy measure for GABA levels due to the previously reported correlation between them (Edden et al, 2009) and ease and speed of testing. The Tilt illusion was used due to its relation with GABAergic properties (Gelbtuch et al, 1986). The lack of findings was not unexpected as the relationship between GABA and gamma is proving to not be as robust as first thought (Couijsen et al, 2015). The lack of relationship is not likely to be due to the task itself as all of the results were indicative of an expected direct effect. This was coupled with a high level of

variation for both the TI and the gamma measures. Although it should be mentioned that Gelbtuch and colleagues did not measure MRS GABA, they inferred an increase in GABA from the administration of Lorazepam, a GABA agonist, which increases the frequency of the opening of the chloride ion channel at the GABAa receptors. This does not necessarily mean there was an increase in in vivo GABA levels in the synapse but more an increase in activity at the post-synaptic neuron. This therefore could account for the lack of relationship between the direct effect and gamma activity in this sample.

8.2.3 Reduction in occipital GABA in patients with a diagnosis of schizophrenia but no difference in sensory motor GABA between groups: This finding supports the work suggesting that whilst GABA appears reduced in certain areas, this does not apply to the brain as a whole (Kegeles et al, 2012; Rowland et al, 2015). The reduction in occipital GABA in this sample was not due to medication effects as olanzapine equivalents were not correlated with GABA levels in the patient group. Further the reduction in GABA being specific to one voxel supports the lack of relationship with medication. If medication was a driving force behind the reduction in GABA then the assumption would be this reduction would occur throughout the brain and would have lead to a difference in the sensory motor region also. Further, the finding of no difference in V1 area, thickness or volume between groups lends further support to the reduction in GABA not being the result of a reduction in gray matter within the patient group.

One possible reason for this pattern of results is that as the patients used in this sample were chronic patients, it could the case that the reduction in occipital

GABA is a biomarker of chronic illness. This question can be answered using a longitudinal design and scanning first episode patients in several different voxels. These same patients would then be followed-up to see how GABA measures within these voxels differ over time and if indeed occipital GABA is a biomarker of chronic illness.

Structural measures were gathered in this experiment but were only analysed in relation to occipital GABA to make sure tissue composition within this voxel did not account for the between group results. This is in line with a recent study by Gaetz, Bloy, Wang, Port, Blaskey, Levy & Roberts (2014) with children on the autism spectrum and typically developing children. They showed reductions in auditory and motor MRS GABA levels in the autistic spectrum children but with no underlying tissue differences. This demonstrates the differences found were not related solely to structural differences between groups, further demonstrated in our study. However, in this thesis it would have been beneficial to have conducted the same structural analysis on the sensory motor voxel also to check for a between voxel as opposed to between group discrepancy in tissue composition accounting for the result.

8.2.4 Reduction in gamma oscillatory activity in patients with schizophrenia using a moving radial stimulus:

The reduction in gamma oscillatory activity was found across both frequency and amplitude for both transient and sustained gamma, although sustained amplitude did not reach significance. This was specific to a radial moving task and did not occur for a static task. This reproduces previous research from Chen and colleagues (2014) who found reductions in gamma amplitude in

patients with schizophrenia. In addition Chen and colleagues related this reduction to levels of cognition using a working memory task whilst Grutzner et al (2013) have related a reduction to perception of Mooney faces.

The lack of a difference between groups when using a static task is interesting, as Chen and colleagues also reported no between group differences in resting gamma amplitude. The difference only occurred when participants were performing a working memory cognitive task. As stated in Chapter 6 Introduction section, the moving radial stimulus requires more attention than the static, thus, the necessary coordination of neural activity needed for strong cognitive performance, thought to be reduced in patients, could account for this result.

The differences between peak frequency and peak amplitude are very interesting in their own right. The reduction in gamma frequency, which is the product of the cortical inhibitory/excitatory interplay, reflects abnormalities in this cycle within the patient group. However, the lack of relationship with GABA levels is again confusing. It could indicate that excitatory processes could be the important aspect of this cycle with inhibitory processes affected indirectly. The STRATA project currently being undertaken at Cardiff, Manchester, Edinburgh and Kings College London is investigating this possibility by measuring excitatory glutamate levels in distinct schizophrenia samples and how this may relate to treatment responsiveness but also gamma measures. Results from this project could indicate if excitation as opposed to inhibition should be considered

The reduction in amplitude in the patient group reflects a reduction in signal, thus a reduction in the synchronised firing of neurons. As synchronised firing is also heavily implicated in perceptual and cognitive processes, this could account for the significant relationship between the psychophysics and imaging measures between sustained gamma amplitude and orientation discrimination thresholds only in the patient group. Conducting the psychophysics within the MEG scanner may have been beneficial in so much as it would have highlighted whether a further reduction in gamma amplitude occurs in the patient group under more cognitively demanding conditions. This could have replicated Chen et al's findings in a much larger sample, and consequently provide clear evidence for a link between levels of cognitive demand and gamma amplitude.

8.2.5 Increase in visually induced gamma frequency and amplitude when using a moving radial stimulus compared to a static stimulus in the patient and control groups:

The increase in gamma activity when using a moving radial stimulus replicated previous research indicating an attentionally engaging and/or a moving stimulus increases both gamma frequency and amplitude in healthy controls (Hoogenboom et al, 2006; Sweetenham et al, 2009). The similar result in the patients is a novel finding and suggests the mechanisms behind this increase are intact but diminished in the patients compared to the controls as evidenced by the reduction in gamma activity across all measures.

As part of the analysis pipeline, high end gamma (80-130Hz) was also drawn from the results but was not analysed fully given time constraints. Given the

extra time needed this analysis could have been interesting as patients have been identified as having reduced gamma in this frequency band when performing Gestalt perceptual tasks using Mooney faces (Uhlhaas et al., 2006). However, high end gamma data should be used with caution as there is a strong possibility that activity in these higher frequency bands are not true results as they can be generated by muscle artefacts such as jaw clenching (Muthukumaraswamy, 2013). This was an additional reason we did not analyse this broadband gamma but in future it would be interesting to see how it compares with low band gamma activity when controlling for such muscle artefacts.

8.2.6 No relationship found between GABA and gamma measures in both patient and control groups:

This finding was not unexpected due to the now mixed results investigating this relationship in healthy controls (Edden et al., 2009; Couijsen et al., 2014). The mixed results in relation to these two measures could involve the methods used to measure GABA. Conventional estimates of GABA, using conventional edited MR sequences that are not macromolecule suppressed, can produce noisy and unreliable results due to macromolecule contamination (Mikkelsen et al., 2015). Although suppressed sequences have poorer signal-to-noise, both methods have comparable levels of repeatability. This offers the opportunity to estimate cleaner GABA measures, which will become increasingly important as more research is conducted using MRS GABA to investigate disease populations.

Specific to this experiment, it could be the case that the lack of relationship was due to GABA measures being at rest and the gamma measures being low level

visual tasks. Again, Chen et al (2014) demonstrated how the relationship between GABA and gamma strengthened when participants were performing a working memory task suggesting a critical role for GABAergic processes in the synchronised oscillatory activity needed for strong cognitive performance. Therefore, a difference in GABA between groups may not have been evident using the resting methods within this thesis. Future work should compare rest levels with levels whilst performing cognitive or perceptual tasks to verify this relationship.

The general lack of relationship between both the imaging and psychophysics was disappointing but was based on correlational analyses which may not have been the appropriate method based on the still relatively small sample size. Interpretation of these results could benefit from analysis using neurophysiologically-informed modeling. For example, Dynamic Causal modeling (DCM) uses spatiotemporal models designed to answer questions about the underlying neuronal architecture and to make inferences about associated neuronal parameters and, in this instance, how these differ between patients and controls, as well as predicting gamma oscillation parameters, GABA levels and performance on the orientation discrimination task (Kiebel, Garrido, Moran, Chen and Friston, 2009). Recently awarded ISSF Wellcome Trust funding will focus on this analysis using this sample.

8.2.7 Patients demonstrate poorer performance in an orientation discrimination task compared to healthy controls:

This finding lends support to the broadening of orientation tuning curves in patients with schizophrenia (Rokem et al., 2011). The mechanisms behind this

however, require further investigation, as occipital GABA levels did not correlate with any of the conditions. This was unexpected as GABAergic processes are strongly implicated in the tuning of orientation curves. It is unlikely in this sample that medication had an effect, as olanzapine equivalents again did not correlate with any of the conditions administered. It could be the case that this study didn't measure the right variable, and other GABAergic properties, such as receptor density, glutamate levels, levels of re-uptake enzymes would be a better indicator of orientation tuning. Alternatively this could lend support to resting in-vivo levels of GABA having little to no effect on tuning curves, but the mechanism behind or ability to pharmacologically manipulate GABA could provide more of an insight into orientation tuning of neurons.

All of the conditions correlated within both the control and patient group suggesting the patients' performance was very repeatable and likely not due to chance.

8.2.8 Patients and controls have comparable magnitudes of direct effects as measured by the Tilt Illusion

This result was unexpected due to previous findings within this thesis of a reduction in occipital GABA levels, patients were expected to have reduced levels of contextual modulation. This did not occur for the TI task with patients demonstrating larger direct effects, albeit not significantly larger, compared to the control group.

The initial hypothesis relating to the TI was based on a pharmacological study so again it may not be baseline in-vivo GABA that is related to performance on

this task, but more the mechanism behind pharmacologically manipulating the levels of GABA that is driving this result. A comparison MRS during a no-drug and drug condition in the same sample would highlight if this is the case.

It, again, is unlikely in this sample that medication had an effect, as olanzapine equivalents again did not correlate with the size of the direct effects. Future work relating this task to GABAergic properties may need to focus on difference aspects of the GABAergic system such as measures of excitation, GABA receptor density etc.

However, the difference between groups being marginal and not reaching significance argues that the patients had a good understanding of the task. This is an important finding as it indicates the TI can be used to gain reliable results from this patient group. The issue with this task based on this thesis is whether it is a reliable proxy measure for cortical inhibition, which given the fact it did not correlate with either imaging measure would suggest it's not. It's future use could come from the fact patients could complete this task well and as such could be used as a control task to ensure patients are completing tasks satisfactorily.

8.2.9 Participants used

The controls used for this study were screened using the MINI interview to rule out current affective and psychotic disorders. In addition all participants were asked to complete a depression and mania questionnaire. Some of the control group demonstrated rather high self-report mania scores using the questionnaires. These participants were not excluded from the experiment as

the variation within the group was quite large and so no one reached +/- 2 SD's from the mean score, which is a widely used cut off point for exclusion of participants. However, it does highlight whether these participants scoring at the higher end of this scale should have been included in the study based purely on these self-report measures.

The patients used in this thesis all had a DSM-IV diagnosis of schizophrenia with no other diagnoses included in any of the experiments. This sample therefore represents a single pathology and is better suited to answering the above questions. However, when looking at the depression and mania scores there is quite a large range of scores. This could have represented different phenotypic subsets within the same diagnosis. Given ample time this thesis could have investigated these potential different subsets separately and how they related to the imaging and psychophysical measures.

The power of this study does need to be considered, as the samples sizes were moderate but not large within this thesis. A-priori power calculations were not performed during this thesis as the time limits imposed on testing made these almost pointless as we simply tested as many people as possible in the time given. For any future studies with less stringent time frames, it would be highly advisable to use a-priori power calculations to inform necessary sample sizes as post-hoc power tests are not advisable due to being simply a one-to-one function of the *p*-value obtained.

8.3 Conclusion

This thesis has demonstrated abnormalities in both in vivo GABA levels and oscillatory activity in the brains of patients with schizophrenia. Through the additional use of psychophysics and the findings of a reduction in performance by patients using an orientation discrimination task, it highlights the possibility and appropriateness of these measures for biomarker development in the future. This is very important as the development of biomarkers can be used in several ways within this patient group; the orientation discrimination task can be developed as an indication of levels of cortical inhibition without the need for a brain scan, these measures can be used as a measure of disease progression, could indicate appropriate future treatments and finally could be used to monitor treatment effectiveness. The orientation discrimination task specifically could be implemented in clinical settings with ease, as they are cheap and simple to administer with outputs easy to interpret.

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Appendices

Appendix 1 -Diagnosis and medication profile of patient group (Chapters 5-7).

Participant	Diagnosis	Olanzapine equivalents (mg)
1	Schizophrenia	20.1
2	Schizophrenia	32.5
3	Schizophrenia	7.5
4	Schizophrenia	15
5	Schizophrenia	0.54
6	Schizophrenia	15
7	Schizophrenia	32.5
8	Schizophrenia	0.24
9	Schizophrenia	0
10	Schizophrenia	1.79
11	Schizophrenia	53.28
12	Schizophrenia	38.4
13	Schizophrenia	15
14	Schizophrenia	13.4
15	Schizophrenia	15
16	Schizophrenia	10
17	Schizophrenia	8.33
18	Schizophrenia	0
19	Schizophrenia	26.8
20	Schizophrenia	21.25
21	Schizophrenia	5.4
22	Schizophrenia	9.99
23	Schizophrenia	1.07
24	Schizophrenia	13.4
25	Schizophrenia	0.71
26	Schizophrenia	0.89
27	Schizophrenia	2.78
28	Schizophrenia	15

Appendix 2 – Descriptive statistics for imaging measures (Chapters 5-7)

	Spike Frequency (Hz)					Spike Amplitude (%)					
	Standard	Radial1	Radial2	Radial3	Radial Average	Standard	Radial1	Radial2	Radial3	Radial average	Sensory motor GABA (iU)
Mean all	*52.574*	*59.000*	*60.179*	*60.273*	*59.734*	*67.546*	*42.764*	*50.295*	*45.709*	*46.144*	*1.295*
St error all	*1.228*	*1.134*	*1.164*	*1.248*	*1.011*	*6.224*	*3.744*	*4.741*	*4.029*	*3.921*	*0.12*
Mean controls	*53.328*	*58.929*	*60.379*	*61.948*	*60.371*	*73.397*	*49.179*	*58.586*	*53.310*	*53.767*	*1.26*
St error controls	*1.449*	*1.467*	*1.708*	*1.652*	*1.463*	*9.043*	*5.451*	*6.811*	*6.074*	*5.648*	*0.12*
Mean Patients	*51.700*	*59.074*	*59.963*	*58.404*	*59.049*	*60.760*	*36.111*	*41.389*	*37.231*	*37.957*	*1.33*
St error patients	*2.096*	*1.790*	*1.637*	*1.886*	*1.434*	*8.624*	*5.012*	*6.396*	*4.923*	*5.183*	*0.14*

	Sustained Frequency (Hz)					Sustained Amplitude (%)					
	Standard	Radial1	Radial2	Radial3	Radial Average	Standard	Radial1	Radial2	Radial3	Radial average	Occipital GABA(iU)
Mean all	*49.843*	*55.936*	*56.313*	*56.282*	*56.086*	*65.167*	*92.582*	*105.134*	*103.782*	*100.287*	*1.740*
St error all	*1.172*	*0.788*	*0.737*	*0.750*	*0.689*	*5.992*	*9.164*	*9.375*	*9.434*	*8.859*	*0.032*
Mean controls	*50.552*	*57.339*	*57.207*	*58.034*	*57.466*	*71.517*	*108.143*	*124.224*	*120.172*	*118.017*	*1.802*
St error controls	*1.410*	*1.142*	*0.951*	*1.100*	*1.026*	*8.755*	*13.853*	*13.604*	*14.352*	*13.001*	*0.040*
Mean patients	*49.020*	*54.481*	*55.352*	*54.327*	*54.605*	*57.800*	*76.444*	*84.630*	*85.500*	*81.244*	*1.667*
St error patients	*1.975*	*1.056*	*1.142*	*0.906*	*0.862*	*8.179*	*11.676*	*12.153*	*11.526*	*11.340*	*0.048*

Appendix 3 - Correlations of MEG imaging and symptom measures (Chapter 6)

		BDI	Altman Mania scale	SAPS	SANS
Transient static amplitude	r value p value	-0.438 .069	0.127 .614	-0.147 .561	0.379 .120
Transient radial amplitude	r value p value	0.044 .829	-0.231 .256	0.169 .400	-0.006 .978
Transient static frequency	r value p value	0.015 .952	-0.001 .995	-0.166 .511	0.99 .696
Transient radial frequency	r value p value	-0.066 .747	0.321 .110	0.349 .075	-.352 .072
Sustained static amplitude	r value p value	-0.268 .282	0.378 .122	-0.043 .864	0.152 .546
Sustained radial amplitude	r value p value	-0.218 .286	0.153 .456	-0.180 .368	-0.334 .089
Sustained static frequency	r value p value	-0.094 .709	-0.041 .872	-0.046 .855	0.276 .268
Sustained radial frequency	r value p value	-0.156 .447	0.091 .657	**-0.510** **.028**	0.113 .574

Bold values represent significant uncorrected r-values at $p<0.05$

Appendix 4 – Descriptive statistics for the psychophysics measures (Chapter 7)

Control group	PSE-difference	OD-250	OD-10	OD-250o	OD-10o
Mean (degrees)	2.4744	0.7490	0.9926	2.6776	3.2693
SE (degrees)	1.8053	0.6756	0.5133	1.6606	1.8988

Control psychophysics descriptive statistics

Patient group	PSE-difference	OD-250	OD-10	OD-250o	OD-10o
Mean (degrees)	3.1986	2.7691	2.9130	9.5000	9.4690
SE (degrees)	2.2585	3.3150	2.5901	6.9081	7.2377

Patient psychophysics descriptive statistics