Modulation of Gamma Oscillatory Activity Through Repetitive Transcranial Magnetic Stimulation in Healthy Subjects and Patients with Schizophrenia

by

Mera Sun Barr

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Institute of Medical Science
University of Toronto

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Abstract

Background: Gamma oscillations (30-80 Hz) in the dorsolateral prefrontal cortex (DLPFC) are associated with working memory; a process involving the maintenance and manipulation of information on line (Baddeley, 1986). Gamma oscillations are supported by gamma-aminobutyric acid (GABA) inhibitory interneurons in the DLPFC. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive method in which to stimulate the cortex that has been shown to modify oscillations, cognition and GABAergic mechanisms. Patients with schizophrenia have severe deficits in working memory that may be related to impairments in GABAergic inhibitory neurotransmission underlying gamma oscillations in the DLPFC.

Objective: First, to evaluate gamma oscillatory activity in patients with schizophrenia during working memory compared to healthy subjects. Second, to examine the effect of rTMS applied over the DLPFC on gamma oscillations generated during working memory in healthy subjects. Third, to examine the effect of rTMS applied to the DLPFC on gamma oscillations in patients with schizophrenia compared to healthy subjects.

Hypotheses: First, it was hypothesized that patients with schizophrenia would exhibit an alteration in gamma oscillatory activity. Second, it was hypothesized that rTMS would be effective in enhancing gamma oscillations in healthy...
subjects. Third, it was hypothesized that rTMS would be effective in inhibiting gamma oscillations in patients with schizophrenia. **Results:** The first study found that patients with schizophrenia generate excessive gamma oscillations during working memory compared to healthy subjects. The second experiment found that rTMS over the DLPFC resulted in the potentiation of gamma oscillations in healthy subjects during working memory. The third experiment demonstrated that rTMS inhibited excessive gamma oscillations in patients with schizophrenia while an opposite effect was found in healthy subjects. **Conclusions:** rTMS applied over the DLPFC modulates frontal gamma oscillatory in healthy subjects and in patients with schizophrenia depending on baseline levels of activity, a finding that may ultimately translate into a better understanding of the mechanisms leading to cognitive improvement in this disorder.
Acknowledgments

My journey to the completion of this degree involved the mentorship, collaboration, support and love of several individuals whom I would like to acknowledge here.

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My life changed the day I met Faranak Farzan. Faranak is probably the brightest woman I know. She possesses a brilliant analytical mind for science that is accompanied by a nurturing heart and soul. Faranak not only renewed my enthusiasm for research, but pushed me to analyze my data critically. Together, commonly known as “Meranak”, we built a strong collaboration and an even stronger friendship. I cherish and carry our friendship with me in my heart. Thankful I am also to the rest of the TMS lab. I feel incredibly lucky to have been a part of such a joyful, fun and productive lab. In particular, I would like to acknowledge Melissa Daigle and Lisa Tran for their critical involvement in my studies. I am also grateful to Ketsa Maceus and Brenda Kirk whom not only provided the utmost care for the patients, but also support and advise that I truly valued. I will really miss the TMS lab. Thank you for all of the wonderful memories.

Thirty years ago, my parents Susan and David Barr, gave me the most precious and beautiful gift. Life, opportunity, and most importantly love. Adopted from a refugee camp in Cambodia, I was exempted from my destiny. I hope that I have made you proud. This work is dedicated to you.
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List of Symbols and Abbreviations

δ  Delta
θ  Theta
α  Alpha
γ  Gamma
β  Beta
5-HT  5-hydroxytryptamine
AC  Anterior Commissure
ANOVA  Analysis of Variance
APB  Abductor Pollicis Brevis
BAs  Brodmann Areas
BOLD  Blood Oxygenation Level Dependent
CAMH  Centre for Addiction and Mental Health
CDS  Calgary Depression Scale
COMT  Cathecol-O-methyltransferase
DLPFC  Dorsolateral Prefrontal Cortex
DLPFC_C  Dorsolateral Prefrontal Cortex on the Cortex
DLPFC_S  Dorsolateral Prefrontal Cortex on the Scalp
DSM  Diagnostic and Statistical Manual of Diseases
eDLPFC_S  Experimental Dorsolateral Prefrontal Cortex on the Scalp
EEG  Electroencephalogram
EPSPs  Excitatory Post Synaptic Potentials
ERD  Event Related Desynchronization
ERS  Event Related Synchronization
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<td>fMRI</td>
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<td>GABA</td>
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<td>GAD 67</td>
<td>Glutamic Acid Decarboxylase</td>
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<td>HS</td>
<td>Healthy Subjects</td>
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<td>IPSPs</td>
<td>Inhibitory Post Synaptic Potentials</td>
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<td>IQ</td>
<td>Intelligence Quotient</td>
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<td>MDD</td>
<td>Major Depressive Disorder</td>
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<td>Magnetoencephalography</td>
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<td>MMRM</td>
<td>Mixed Model Repeated Measures</td>
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<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>NMDA</td>
<td>N-Methyl-D-Asparate</td>
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<tr>
<td>NTC</td>
<td>Non Target Correct</td>
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<td>P</td>
<td>Probability</td>
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<td>PANSS</td>
<td>Positive and Negative Symptom Scale</td>
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<td>Personality Assessment Screener</td>
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<td>SANS</td>
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<td>SAS</td>
<td>Statistical Analysis System</td>
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<td>Schizophrenia</td>
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<td>SD</td>
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<td>SICI</td>
<td>Short Interval Cortical Inhibition</td>
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<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
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<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
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<tr>
<td>TC</td>
<td>Target Correct</td>
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<td>tDCS</td>
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Chapter 1

1 Schizophrenia

1.1 Schizophrenia: Phenomenology

Schizophrenia is still one of the most serious and debilitating mental disorders of modern medicine. The classification of schizophrenia has a long history that ranges from narrow to broad definitions based on both abstract theories and empirical data. Schizophrenia was first classified by Bendict Morel in 1853. Morel described schizophrenia as a syndrome which affects young adults which he referred to as démence précoce. The classification of psychoses was later significantly advanced by Emil Kraepelin (1856-1926) and is still influential today. In 1898, he proposed the term dementia praecox (premature dementia) for schizophrenia based on longitudinal studies of the patients’ conditions over time. In Kraepelin’s description, dementia praecox has an early onset that progressively deteriorates to a common end stage. Despite the dismal outcome described by Kraepelin, he acknowledged the need for cross-sectional diagnostic guidelines including bizarre thought disturbances, delusions, auditory or other hallucinations, catatonia, abnormalities in volition and “a pervasive reduction in cognitive and affective capacity”, in addition to, “peculiar states of mental weakness”. Importantly, Kraepelin believed that patients with dementia praecox do not lose metal abilities but rather lose the ability to use them appropriately. Kraepelin’s definition of schizophrenia was later challenged by Eugen Bleuler (1857-1939) for being too narrow and described only the severe cases. Instead Bleuler’s definition included a more heterogeneous grouping that emphasized the loss of mental connectedness in which then he came to name this disease as schizophrenia. According to
Bleuler, associative splitting is present in the fundamental symptoms of schizophrenia that included: (1) association defects; (2) autism; (3) ambivalence; and (4) disturbance of affect. Bleuler’s categorization of schizophrenia was widely accepted and used by clinicians until the publication of the Diagnostic and Statistical Manual of Diseases (DSM-II) in 1980. According to the fourth edition of the DSM; there are five subtypes of schizophrenia: 1) Paranoid; 2) Disorganized; 3) Catatonic; 4) Undifferentiated; and 5) Residual. Despite the widespread use of the DSM-IV as a diagnostic tool, scientists and clinicians remain skeptical of disease categories and are in favour of a more mechanistic basis of brain diseases.

Schizophrenia is characterized by three main categories of symptoms: positive and negative symptoms and cognitive deficits. Positive symptoms include auditory hallucinations, bizarre delusions and thought disorder, while negative symptoms include blunted affect, alogia, and anhedonia. Cognitive deficits in patients with schizophrenia have been shown across a wide variety of cognitive tasks including executive functioning, attention, memory, working memory and general intellectual functioning (Weickert, Goldberg et al. 2000). Antipsychotic medications are effective in managing positive symptoms however little improvement has been shown on negative symptoms and cognitive deficits.

1.2 Schizophrenia: Epidemiology and Costs to Society

A recent review of epidemiological data suggests that the lifelong prevalence and incidence of schizophrenia is between 0.30-0.66% and 10.2-22.0 per 100 000 persons per year (McGrath, Saha et al. 2008). In Canada, the estimated number of persons with schizophrenia was 234 305 in 2004 (Goeree, Farahati et al. 2005). However these numbers depend on the criteria used to define schizophrenia (Castle, Wessely et al. 1993). Symptoms typically begin during late adolescence and may differ between genders. That is, there are reports of males developing symptoms earlier
than females although this has not been consistently shown across countries and at all ages of onset (Jablensky 2000). The outcome of schizophrenia has been shown to improve with follow up care by approximately 60% (Harding, Brooks et al. 1987) and reduce relapses through combined antipsychotic medication and psychosocial rehabilitation (Hogarty 1993). Furthermore, studies have shown that outcome in industrialized countries is typically worse as compared to third world countries (Kulhara and Chandiramani 1988; Ohaeri 1993). For example, according to a World Health Organization study of 10 countries, significantly better outcomes were found in Nigeria, India and Colombia compared to the United States, Japan and England (Jablensky 2000).

Financial costs to society are substantial due to the chronic nature of schizophrenia and its early onset. Globally, the direct costs of schizophrenia on healthcare budgets for the costs of prescription medications, hospitalizations, diagnoses, procedures and long-term care services are approximately 1.5-3% of the total national healthcare expenditure (Knapp 1997; Wu, Birnbaum et al. 2002; Andlin-Sobocki and Rossler 2005; Chisholm, Gureje et al. 2008). The indirect costs of schizophrenia are even higher than the direct costs including increased unemployment, reduced workplace productivity, caregiver burden, and premature mortality from suicide (Knapp 1997; Goeree, Farahati et al. 2005; Chang, Cho et al. 2008). Patients with schizophrenia also have high rates of physical comorbidity including diabetes, hyperlipidaemia, cardiovascular disease and obesity further substantiating these costs (Lambert, Velakoulis et al. 2003). In the United States, the overall cost of schizophrenia was estimated to be $62.7 billion in 2002 of which $22.7 billion comprised of direct cost in addition to $7.6 billion of indirect cost (Wu, Birnbaum et al. 2002). In the United Kingdom, £810 million was incurred by persons with schizophrenia equaling 2.76% of total National Health Service expenditure in 1992-1993 (Knapp 1997). In Canada, the burden of schizophrenia on health care was estimated to be $2.02 billion in
2004. Additionally costs of employment rate, productivity morbidity, and mortality loss was $4.83 billion for the total cost of schizophrenia of $6.85 billion in 2004 (Goeree, Farahati et al. 2005). Finally, the landmark World Bank and World Health Organization study *The Global Burden of Disease*, ranked schizophrenia 5th among the leading causes of disability measured by Disability-Adjusted Life Years in industrialized countries and 9th worldwide (Murray and Lopez 1996).

1.3 Schizophrenia: Etiology

The pathophysiology underlying schizophrenia is still not completely understood. An interaction between genetic predisposition and several environmental risk factors however has been suggested to contribute to the development of this disease.

1.3.1 Genetic and Environmental Risk Factors

Schizophrenia is a familial disease despite the fact that more than eighty percent of persons with schizophrenia have no affected relatives within the first degree of kinship and more than sixty percent have families without a history of this disorder (Nasrallah and Smeltzer 2002). Studies in monozygotic twins indicate that the concordance rate is approximately fifty percent (Kaplan, Sadock et al. 1994). In addition, family and twin studies suggest that in first-degree relatives and dizygotic twins the risk for developing schizophrenia is estimated to be ten-fifteen percent (Kendler and Robinette 1983; McGuffin, Farmer et al. 1984; Kaplan, Sadock et al. 1994). Adoption studies have also yielded interesting results showing an increased risk of developing schizophrenia in children of schizophrenic birth parents adopted into non-schizophrenic homes (Heston 1966); while children with non-schizophrenic parents adopted into schizophrenic families do not (Wender, Rosenthal et al. 1977).
Over the past twenty years, identification of genetic linkage using DNA polymorphism technology (Botstein, White et al. 1980) has now been applied to schizophrenia. In this regard, linkage studies have identified loci on chromosomes 1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 15, 18, 22, and X implicated at the Sixth World Congress on Psychiatric Genetics (Brzustowicz, Hodgkinson et al. 2000) (Barden and Morissette 1999; Craddock and Lendon 1999; Crowe and Vieland 1999; Curtis 1999; Detera-Wadleigh 1999; Gejman 1999; Hallmayer 1999; Kennedy, Basile et al. 1999; Nurnberger and Foroud 1999; Paterson 1999; Schwab and Wildenauer 1999; Van Broeckhoven and Verheyen 1999; Wildenauer and Schwab 1999; Riley and McGuffin 2000). However, such findings should be replicated as these studies have produced conflicted reports or did not satisfy statistical criteria for significant linkage (Lander and Kruglyak 1995). These studies though identified such chromosome loci by genome scanning methods that were not hypothesis driven. Although there is a clear association between altered dopamine activity in schizophrenia, studies have failed to establish a link between dopamine receptors or transporter genes with this disorder (Coon, Jensen et al. 1994; Hallmayer, Maier et al. 1994; Maier, Schwab et al. 1994; Nanko, Fukuda et al. 1994; Ravindranathan, Coon et al. 1994). There has been however reports of variations in the cathecol-O-methyltransferase (COMT) gene (Egan, Goldberg et al. 2001) and varied reports with 5-hydroxytryptamine (serotonin; 5-HT) receptor genes (Williams, Spurlock et al. 1996; Owen 2000) in the frontal cortex associated with increased risk for the development of schizophrenia.

An area on chromosome 22q11-13 has been linked to psychosis and schizophrenia. More specifically, abnormalities in chromosomes 5q, 11q, 18q, 19q, and 22q have been associated with both psychosis and schizophrenia (Bassett 1992). The 22q11.2 area is subject to deletions that have been associated with the DiGeorge and velocardiofacial syndromes (Amati, Conti et al. 1999) and increased risk of schizophrenia (Bassett, Chow et al. 2000). DISC-1 is another gene
that has been shown to be disrupted in patients with schizophrenia in Scottish (Millar, Wilson-Annan et al. 2000; Millar, Christie et al. 2001) and Finnish (Ekelund, Hovatta et al. 2001) families although the function of this gene has not been well characterized. To date, chromosomal and gene abnormalities associated with schizophrenia have yielded some interesting results, however, these findings are not selectively linked to schizophrenia as they overlap with other psychiatric disorders. Moreover, current diagnoses have yet to establish biological validity or validation and the identification of phenotypes have not been simplified by genetic association (Bertolino and Blasi 2009).

In addition to the genetic predisposition of schizophrenia, several environment risk factors have been identified that contribute to the development of this disorder. Moreover, the time in which individuals are exposed to such environmental risk factors is important. For example, complications during pregnancy, reduced vitamin D levels, increased stress to the mother, diabetic mother, and those mothers older than 35 years of age during the prenatal stage have been shown to contribute to the development of schizophrenia (McGrath 1999; Cannon, Jones et al. 2002; Wohl and Gorwood 2007; Khashan, Abel et al. 2008). Post-natal environmental risk factors such as fetal hypoxia and babies born at higher altitudes have been reported to increase the probability of developing schizophrenia (Krabbendam and van Os 2005; McGrath, Saha et al. 2008). Exposure to other environmental risk factors such as ethnicity, urbanization, and cannabis use during childhood to young adulthood has also been identified in the development of schizophrenia (Krabbendam and van Os 2005; McGrath, Saha et al. 2008; Veling, Mackenbach et al. 2008).

The search for endophenotypes of interest for specific genetic factors in schizophrenia has been considered. Notably, cognitive deficits may represent endophenotypes which are intermediate
phenotypes that have been suggested to provide a more reliable index of liability than the illness itself (Gottesman and Shields 1972). In this regard, a meta-analysis performed on unaffected first-degree relatives of schizophrenia patients compared to healthy subjects demonstrated significant group differences in performance across attention/working memory, verbal memory, visual memory, executive function, spatial ability, motor function, language function, and general intelligence cognitive domains (Snitz, Macdonald et al. 2006). This study in addition to previous meta-analyses (Grove, Lebow et al. 1991; Cannon, Zorrilla et al. 1994; Park, Holzman et al. 1995; Faraone, Seidman et al. 1996; Cosway, Byrne et al. 2000; Curtis, Calkins et al. 2001; Appels, Sitskoorn et al. 2003) demonstrate that cognitive deficits, notably in executive control functions, may serve as valuable endophenotypes of interest in search for specific genetic factors related to schizophrenia.

1.4 Neurobiology of Schizophrenia

1.4.1 Neurochemistry

1.4.1.1 Dopamine

Over the last 5 decades, the involvement of dopamine in the pathophysiology and treatment of schizophrenia has been an area of immense research. Dopamine activates 5 types of metabotropic G protein-coupled receptors in the brain: D1, D2, D3, D4 and D5 (Neve, Seamans et al. 2004). Dopamine is present in the ventral tegmental area of the midbrain, substantia nigra, and hypothalamus. Moreover, dopamine is involved in the control of information flow in the frontal lobe to other areas of the brain. Dopamine serves several important functions in the brain including motivation, sleep, mood, voluntary movement and working memory (Jones, Kilpatrick et al. 1986). The dopamine hypothesis first proposed that hyperactive dopamine neurotransmission underlies the presentation of positive symptoms of schizophrenia (Carlsson
This hypothesis was based on the relationship between the dose of antipsychotic medication and their potency to block dopamine D2 receptors (Seeman and Lee 1975; Creese, Burt et al. 1976) and also by the psychotogenic effects of dopamine-enhancing drugs (Angrist and van Kammen 1984; Lieberman, Kane et al. 1987). This classical formulation of the dopamine hypothesis mostly emphasized subcortical regions including the striatum and the nucleus accumbens based on the prevalence of dopamine terminals and D2 receptors.

Although the classical dopamine hypothesis satisfied the relationship between dopamine and positive symptoms, it did not account for persistent negative and cognitive symptoms with antipsychotic treatment. Moreover, the classical dopamine hypothesis did not explain the relationship between genetics, neurodevelopment deficits or other known risk factors for schizophrenia (Howes and Kapur 2009). In this regard Davis et al (1991) published a seminal paper on the reformulation of the classical dopamine hypothesis that was based on more recent evidence from neuroimaging, post-mortem, and animal studies, which placed the emphasis on the interaction between hypodopaminergic activity in the prefrontal cortex and hyperdopaminergic activity in more subcortical regions (Davis, Kahn et al. 1991). For example, neuroimaging studies have suggested that an alteration in prefrontal cortical functioning may underlie negative and cognitive symptoms in this disorder (Knable and Weinberger 1997).

Evidence from preclinical studies has demonstrated the importance of dopamine neurotransmission at D1 receptors, which is the predominant dopamine receptor in the neocortex, in the proper functioning of the prefrontal cortex (Goldman-Rakic, Muly et al. 2000). In humans it has been shown that carriers of high-activity allele of COMT, an enzyme involved in the metabolism of dopamine, perform worse on cognitive tasks compared to those carriers of the allele that induces lower concentration of dopamine in the prefrontal cortex (Goldberg and Weinberger 2004). Finally, clinical studies also support this association revealing a relationship
between reduced cerebrospinal fluid homovanillic acid with indexes low dopamine activity in the prefrontal cortex and poor performance on working memory tasks (Weinberger, Berman et al. 1988; Kahn, Harvey et al. 1994). This modified dopamine hypothesis therefore proposed that positive symptoms arise from striatal hyperdopaminergia while prefrontal hypodopaminergia may underlie negative and cognitive symptoms in schizophrenia.

The second reconceptualization of the dopamine hypothesis was criticized for its lack of direct evidence for reduced dopamine in the frontal cortex and limited direct support for hyperactive dopamine in the striatum (Howes and Kapur 2009). In this regard, Howes and Kapur recently published a third version of the dopamine hypothesis in 2009 that accounts for recent evidence of known risk factors in addition to sociocultural factors that together contribute to schizophrenia (Howes and Kapur 2009). This hypothesis proposed 4 distinctive components. The first component proposes that several “hits” including fronto-temporal dysfunction, genes, stress, and drugs contribute to dopamine dysregulation referred to as the final pathway to psychosis in this disorder. The second component places less emphasis on the D2 receptor level to the level of presynaptic dopaminergic control level contributing to the dopamine dysregulation. The third component relates dopamine dysregulation to psychosis rather than leading to schizophrenia itself. That is, the specific diagnosis is a result of the interaction of hits that interact with sociocultural contributions. The final fourth component hypothesizes that dopamine dysregulation alters the evaluation of stimuli possibly through aberrant salience (Howes and Kapur 2009).

1.4.1.2 Serotonin

Serotonin has also been examined in schizophrenia and is targeted in newer antipsychotic medication. Serotonin neurotransmission has been implicated in the regulation of mood and
suicidal tendencies across neuropsychiatric disorders (Bellivier, Szoke et al. 2000). Serotonin is released predominantly from the raphe nuclei in the brain stem and is produced from the amino acid L-tryptophan by tryptophan hydroxylases2 (Grohmann and Trendelenburg 1988). In the lower raphe nuclei, axons innervate the cerebellum and the spinal cord while the axons from the upper raphe nuclei innervate the entire brain (Grohmann and Trendelenburg 1988). There are at least 14 different subtypes of serotonin receptors that have been further classified into 7 subfamilies of which are mostly part of the G-protein linked receptors that exert both excitatory and inhibitory cell responses (Wong and Van Tol 2003). Serotonin receptor activity involves the signaling through several different mechanisms including Gi/o which inhibit adenylyl cyclase, PCL-β, and adenylyl cyclase and is expressed in many different peripheral tissues as well as the central nervous system (Raymond, Mukhin et al. 2001). An alteration in serotonin activity in schizophrenia was first proposed owing to the evidence of its similar chemical structure with the hallucinogenic drug lysergic acid diethylamide (LSD; (Gaddum 1954); (Wooley and Shaw 1954)). This has since been supported by post-mortem and neuroimaging studies which have reported an alteration in serotonin activity in patients with schizophrenia. For example, post-mortem studies consistently demonstrate increased levels of 5-HT₁A receptor subtypes examined through 5-HT₁A receptor agonist [³H]8-hydroxy-2-[di-n-propyl-amino]tetralin([³H]8-OH-DPAT) in the prefrontal cortices of patients with schizophrenia (Hashimoto, Nishino et al. 1991; Joyce, Shane et al. 1993; Simpson, Lubman et al. 1996; Sumiyoshi, Stockmeier et al. 1996; Gurevich and Joyce 1997). Reductions in the density of 5-HT transporters labeled with [³H]paroxetine (Laruelle, Baldwin et al. 1993), [³H]cyanoimipramine (Joyce, Shane et al. 1993), and with [³H]citalopram (Ohuoha, Hyde et al. 1993) have also been shown through magnetoencephalography (MEG) in the frontal cortices of patients with schizophrenia compared to healthy subjects and other brain regions. Decreases in 5-HT₂A density have also been reported
although these findings may be confounded by medication effects. Together these studies suggest an alteration in serotonin activity in the prefrontal cortex of patients with schizophrenia, however such abnormalities are not as influential as the evidence for an impaired dopamine system in this disorder.

1.4.1.3 Glutamate

Excitatory neurotransmission is predominantly mediated by the neurotransmitter glutamate. Glutamate is involved in several cognitive functions such as learning, memory, and synaptic plasticity (Johnson 1972). Glutamate is synthesized locally or as a product of the Krebs cycle and binds to both ionotropic and metabotropic receptors. For example, glutaminergic excitatory neurotransmission is mediated by three main types of ionotropic receptors: N-methyl-D-aspartate (NMDA), amino-hydroxy-5-methyl-4-isoxazole propionic acid and kainic acid (Newcomer and Krystal 2001). The glutamate hypothesis of schizophrenia was based on the finding of increased psychotic symptoms in healthy subjects following the channel blocking of NMDA receptors and the exacerbation of positive and cognitive symptoms in patients with schizophrenia (Luby, Cohen et al. 1959; Tamminga 1998; Krystal, D'Souza et al. 1999). Animal studies have demonstrated that reduced levels of NMDA receptor subunit results in behavioural changes similar to symptoms of schizophrenia including hyper locomotion and social withdrawal in mice. Moreover, such induced behavioural changes were ameliorated by antipsychotics (Mohn, Gainetdinov et al. 1999). In addition, evidence of glutamate hypofunctioning has also been revealed through neuroimaging studies in patients with schizophrenia during rest or while performing cognitive tasks. More recent studies then suggested that the noncompetitive NMDA receptor antagonists were found to preferentially increase metabolism and extracellular glutamate levels localized in the limbic circuits rather than throughout the entire cortex (Lahti, Koffel et al. 1995; Moghaddam, Adams et al. 1997; Duncan, Miyamoto et al. 1999; Holcomb,
Lahti et al. 2005). Although these studies do not suggest a generalized reduction in NMDA receptor activity throughout the entire brain, it is clear that impaired NMDA function that is critical to limbic forebrain functioning is involved in the pathophysiology of schizophrenia (Marek, Behl et al.). Furthermore, genetic studies have failed to find a link between schizophrenia and NMDA genes. Finally, glutamate is also involved in inhibitory neurotransmission serving as a precursor in the synthesis of gamma-aminobutyric acid (GABA), a mechanism that has also been shown to be impaired in patients with schizophrenia and will be reviewed in the following section.

1.4.1.4 GABA

Altered inhibitory neurotransmission has also been implicated in the pathophysiology of schizophrenia (Roberts and Frankel 1950). Inhibitory neurotransmission is a process that involves the suppression of cortical activity and is predominantly mediated by GABA that is present in all cortical layers of the brain (Jones 1993). GABA is almost exclusively synthesized by the excitatory neurotransmitter glutamate and its effects are mediated by ionotropic GABA$_\text{A}$ and metabotropic GABA$_\text{B}$ receptors. GABA$_\text{A}$ receptor activity is reliant on the concentration of cation-chloride while activity of GABA$_\text{B}$ receptors depends on the presence of calcium and magnesium. GABA$_\text{B}$ receptors are located mainly in the presynaptic membrane of aminergic synapses and when GABA or GABA agonists bind to these receptors, the release of dopamine, noradrenergic, and serotonin is inhibited. The GABAergic hypothesis of schizophrenia was introduced shortly after discovery of GABA and its inhibitory role in the central nervous system (Roberts and Frankel 1950; Roberts 1972).

Impaired GABAergic inhibitory neurotransmission in schizophrenia has been specifically shown in the dorsolateral prefrontal cortex (DLPFC). For example, post-mortem studies report
reductions of the messenger RNA for 67 kiloDalton isoform of glutamic acid decarboxylase (GAD 67), an enzyme that synthesizes GABA, in 25-30% of GABAergic interneurons in the DLPFC (Akbarian, Kim et al. 1995; Volk, Pierri et al. 2002; Hashimoto, Volk et al. 2003). Furthermore, decreased densities of interneurons in layer II of the prefrontal cortex of schizophrenia patients has also been reported (Benes, McSparren et al. 1991). Reductions in the synthesis of GABA have also been demonstrated in GABAergic neurons that express calcium binding protein parvalbumin in the DLPFC of patients with schizophrenia (Lewis, Hashimoto et al. 2005). Such deficits in GABAergic activity in patients with schizophrenia has been suggested to result from excessive activation of dopamine D₂ receptors resulting in heightened excitability in the cortex possibly leading to psychotic symptoms (Benes 1997).

1.5 Cognitive Impairment

Cognitive deficits are now considered a core component in schizophrenia (Elvevag and Goldberg 2000). Deficits in cognition are estimated to occur in 75-85% of patients with schizophrenia and often are presented prior to the onset of other symptoms (Reichenberg, Weiser et al. 2006). Despite the effectiveness of antipsychotic medication for the management of positive symptoms, cognitive impairment persist (Heinrichs 2005). Cognitive impairment has been shown as the best predictor of long term functional outcome compared to indices of the positive or negative symptom domain (Green 1996; Gold 2004). Relationships have been demonstrated, though, between cognitive performance and negative symptoms (Harvey, Howanitz et al. 1998; Park, Puschel et al. 1999). Moreover, it has been argued that schizophrenia patients with the greatest cognitive impairment present the most prominent negative symptoms (Basso, Nasrallah et al. 1998; Villalta-Gil, Vilaplana et al. 2006). It has further been suggested that negative symptoms
mediate the relationship between cognition and functional outcome (Ventura, Hellemann et al. 2009).

Patients with schizophrenia generally perform one standard deviation below the mean on tests of cognition (Weickert, Goldberg et al. 2000; Fioravanti, Carlone et al. 2005). A meta-analysis conducted by Fioravanti et al 2005 analyzed studies that examined cognitive functioning across the traditional domains of cognition that includes the assessment of: 1) global cognitive functioning or IQ; 2) memory functioning; 3) language; 4) executive functioning; and 5) attention in patients with schizophrenia compared to healthy subjects. The results of the meta-analysis revealed that patients with schizophrenia perform worse than healthy subjects across all cognitive domains (Fioravanti, Carlone et al. 2005). Moreover, the greatest significant differences between the two groups were found in memory, language and IQ. Fioravanti et al (2005) therefore confirming previous studies (i.e., (Heinrichs and Zakzanis 1998) demonstrating a generalized cognitive deficit in patients with schizophrenia compared to healthy subjects. Importantly this study acknowledges sources of heterogeneity such as patient characteristics (i.e., duration of illness, medication) and also generic differences such as the lack of motivation. Despite these factors that could have contributed to within patient group differences, nevertheless, it is evident from studies conducted from 1990-2003 that patients with schizophrenia are impaired across all cognitive domains (Fioravanti, Carlone et al. 2005).

1.5.1 Summary

Schizophrenia is a complex neuropsychiatric disorder that presents positive, negative, and cognitive deficits as part of its symptom profile. These symptoms have been linked to abnormalities in dopamine, GABA, and other neurotransmitter systems. Furthermore, there is considerable evidence for a genetic component in schizophrenia however interactions with
external environmental risk factors have also been identified. Cognitive impairment in particular has been demonstrated as the best predictor of long term functional outcome and therefore such deficits are well studied in this disorder. Working memory is a higher-order cognitive process that involves the maintenance and manipulation of information on line (Baddeley 1986). This process is involved in many cognitive tasks and is important in everyday functioning. There is considerable evidence for working memory impairment in patients with schizophrenia therefore underscoring the need for a better understanding of the mechanisms underlying this cognitive process. The proceeding sections will review this literature on the underlying mechanisms of working memory and evidence for impaired working memory function in patients with schizophrenia.

1.6 Working Memory

Working memory is commonly defined as the ability to temporary retain information that no longer exists in the external environment or is retrieved from long-term memory (D'Esposito 2007). Such internal representations are short-lived, but can be stored for longer periods of time through the active maintenance or rehearsal strategies, and can be also subjected to various operations that manipulate the information for goal-directed behaviour (D'Esposito 2007). Working memory is involved in everyday functioning such as calculating the tip amount at a restaurant and is critical to other cognitive abilities such as reasoning, language comprehension, planning and spatial processing (D'Esposito 2007). To this end, working memory has been proclaimed as “perhaps the most significant achievement of human mental evolution” (Goldman-Rakic 1992).

Working memory is a theoretical construct that is used in the fields of cognitive psychology and cognitive neuroscience and each discipline has defined working memory through several
different models (for review, (Miyake and Shah 1999)). The first and most influential proposal of working memory was put forth by Baddeley and Hitch in 1974 that involved multiple components to mediate executive control and the active maintenance of temporarily stored information. In Baddeley’s working memory model, four components were proposed in which two of these components serve as storage buffers for the maintenance of visual and verbal information referred to as the visuo-spatial sketchpad and the phonological loop (Baddeley and Hitch 1974). A third episodic buffer was then included in this model for the storage of information in a multi-dimensional code and serves as an interface between the visual and verbal buffers and the fourth component referred to the central executive component (Baddeley 2000). The central executive component is then hypothesized to guide the manipulation and transformation of information held within the storage buffers (Baddeley and Hitch 1974).

A critical component of Baddeley’s working memory model is the existence of verbal and spatial storage buffers. Moreover, the cognitive concept of a buffer indicates that the temporary storage of verbal and spatial information requires the transfer of information from one brain region to another (D'Esposito 2007). In this regard, a wealth of studies investigating the neural correlates of working memory consistently report the involvement of a number of cortical regions, particularly regions within the prefrontal cortex, anterior cingulate area, and posterior parietal cortex (Jonides, Schumacher et al. 1998; Fletcher and Henson 2001; Leung, Gore et al. 2002; Sakai, Rowe et al. 2002). It has been shown that different brain regions are activated while performing working memory tasks that are dependent on the task and therefore may be related to task-specific processing or the modalities in which the task employs. However, among these brain regions the DLPFC (Brodmann areas (BAs) 9, 46, and 9/46) is consistently shown to be activated across working memory tasks and has been considered for the role of the central executive. Moreover, the central executive is believed to be involved in the manipulation of
information being stored in the domain-specific buffers, sustained attention during competing information, temporal coding or sequencing, updating the contents of working memory, and the maintenance of goal representations in working memory (Barch and Smith 2008). Other brain regions however have also been implicated to play the role of the central executive in the development of more recent working memory theories.

There are several competing working memory theories that have been proposed since the time of Baddeley with the advancement in neuroimaging. For example, Wager and Smith (2003) conducted a meta-analysis on neuroimaging working memory studies that recruited a central executive showed activation in the posterior parietal cortex (Wager and Smith 2003). By contrast, Jonides et al (1998) demonstrated increased processing in the left prefrontal cortex while performing a verbal working memory task measured with positron emission tomography (PET) in healthy subjects (Jonides, Schumacher et al. 1998). Such inconsistent results may be attributed to the type of working memory or the level of task difficulty tested. In this regard, the involvement of the DLPFC and ventrolateral prefrontal cortex in the mediation of working memory across different types of information being processed (i.e., spatial versus non-spatial) has led to two conflicting views for the functional specialization of the lateral prefrontal cortex. In the first view, the lateral prefrontal cortex is specialized according to the type of information being processed (Levy and Goldman-Rakic 2000). That is, spatial information is mediated through the DLPFC, while non-spatial information is mediated by the ventrolateral prefrontal cortex consistent with the dorsal “what” and ventral “where” visual pathways (Curtis and D'Esposito 2004). By contrast, the second view suggests a process-specific model for working memory that emphasizes the importance of the level of processing required for the task rather than the sensory modality (Petrides 1995; Petrides 2000). In this model, a hierarchy suggests that the ventrolateral prefrontal cortex is involved in the active encoding and retrieval of information,
while the mid-dorsolateral prefrontal cortex supports higher order executive control functions such as the monitoring and manipulation of stored information (Curtis and D'Esposito 2004).

Alternatively, working memory has been considered as a process within long-term working memory rather than a separate mechanism. For example, Cowan’s model argues against devoted brain region or buffers specialized to mediate different information contained in working memory; and instead, suggest that the information contained in working memory activates a portion of long-term memory that is currently being attended to (Cowan 1997). Support for Cowan’s model is starting to emerge with evidence from neuroimaging studies. For example, work by the D’Esposito group (Druzgal and D'Esposito 2003; Ranganath, Cohen et al. 2004) have demonstrated different activation depending on the type of stimuli being maintained in working memory thereby supporting the notion that the neural systems that process the information currently being focused on in working memory should activate the same systems used to process initially or store information in long-term memory. Cowan’s model of working memory is similar to one put forth by Engle and colleagues (Kane and Engle 2000). In this model of working memory, Engle’s group emphasizes the centrality of goal maintenance and interference control of working memory. This body of work suggests that an important aspect of working memory is the ability to maintain goal representations that allow one to select task-relevant information from task-irrelevant information in the protection of information that may be distracting or interfering. In this view, working memory is regarded as controlled attention. Moreover, this conceptualization of working memory explains the relationship between working memory capacity and variation in performance on working memory tasks such as the antisaccade and the Stroop which are considered goal maintenance tasks (Kane and Engle 2003; Unsworth, Schrock et al. 2004).
1.6.1 Summary

Since Baddeley’s first proposal, a number of different theories of working memory have emerged. Such theories, however, conflict with regards to their emphasis on a unitary nature of working memory (Engle, Cantor et al. 1992), whereas others emphasize the non-unitary nature of working memory and argue for a more domain-specific view of working memory (e.g. (Daneman and Tardif 1987)). In addition, others have emphasized the individual differences in working memory capacity and are conceptualized in terms of variation in the total amount of mental resources available (e.g. (Just and Carpenter 1992)); however, these theories are conflicted by those that claim that long-term knowledge and skills may attribute to individual differences in working memory capacity (e.g. (Ericsson and Kintsch 1995)). Despite the number of theories regarding working memory, it is evident that many brain regions are involved in this process with the DLPFC being the most important.

1.6.2 Assessment of Working Memory

1.6.2.1 Neuropsychological Assessment

Several neuropsychological tests are used to assess working memory and are typically administered as part of a cognitive battery, such as the Wechsler Adult Intelligence Scale, and the MATRICS battery. Neuropsychological tests assess working memory across sensory domains including non-verbal (e.g., visual, spatial, visuospatial, audiospatial and executive) and verbal (sometimes referred to as phonological). For example, the delayed match to sample task can test visual, spatial, visuospatial, auditory, audiospatial working memory in which the stimulus is briefly presented to the subject followed by a delay period. Another stimulus is then presented and the subject is required to determine if the current stimulus was the same as the one previously presented. An example of a spatial working memory task is one in which 10 cubes are irregularly spaced on a board and the subject is required to tap the cubes in the same or reverse
order as the test administrator. Furthermore, the number of blocks that the subject is required to remember is increased with each trial thereby increasing the cognitive demand or working memory load. An example of an executive working memory task is the self-ordered pointing task in which subjects are presented a number of images or words which are arranged on a display. Several trials are subsequently presented each with a different arrangement that contain some of the previous stimuli and subjects are required to point to stimuli that they had not pointed to in the previous trials. By contrast, a test to assess verbal working memory is the number-letter-span in which a list of both numbers and letters is read out and the subject is required to mentally reorder the string of numbers and repeat them to the administrator. The number-letter-span also increases in working memory load with each successful trial. Another common assessment of working memory is the classic Sternberg test. In the Sternberg task, lists are presented with varying numbers of letters or words followed by a delay or retention period. Following the retention period, a probe then is presented and the subject is required to answer yes or no if the probe was on the list. The final working memory test that will be reviewed in this section is the N-back task. In this task, letters are presented one at a time and subjects are required to determine if the same letter was presented “N” trials back. Typically, this task is administered at increasing working memory loads (e.g., 0-3 back); however, the validity of the 0 and the 3-back has been debated. That is, in the 0-back condition there is no memory component and therefore has been criticized for serving as a control condition for this task and may only reflect attentional components. The 3-back task condition has also been debated for its validity due to the ability of subjects to successfully perform this task typically resulting in an inverted u-shaped curve in healthy subjects (Callicott, Mattay et al. 1999). For example, it has been suggested that the 3-back may involve attentional components (Kane, Conway et al. 2007) and/or short term memory aspects of working memory (Shelton, Metzger et al. 2007). The drawback of the N-back task is
that the different subprocesses of working memory such as encoding, maintenance, and retrieval cannot be examined. Finally, the N-back task is different from the other working memory tasks reviewed because it involves continuous updating from trial to trial.

1.6.2.1.1 Neuropsychological Evidence for Working Memory Deficits in Schizophrenia

Goldman-Rakic (1994) proposed a neuropsychological model of schizophrenia with working memory impairment as the core feature. Moreover, Goldman-Rakic specifically related working memory dysfunction to a formal thought disorder which she argued was characterized by a failure to adequately retain recent information or ideas in working memory. There has been however some debate as to whether the putative neuropsychological impairment in schizophrenia is a generalized one (Blanchard and Neale 1994) or rather certain functions are differentially impaired. That is, increased distractibility (Oltmanns, Ohayon et al. 1978), memory impairment (McKenna, Tamlyn et al. 1990), attentional disturbance (Nuechterlein and Dawson 1984) and executive function (Weinberger, Berman et al. 1986) have all been suggested as core features of schizophrenia. It is difficult though to distinguish impaired neuropsychological functions from one another as these tests tend to vary in their degree of difficulty and sensitivity to the effects of impaired functioning (Chapman and Chapman 1973) in addition to differences in intellectual abilities among patients with schizophrenia (Forbes, Carrick et al. 2009). In this regard, Silver et al (2003) tested the hypothesis that impaired working memory is a core deficit underlying multiple neuropsychological deficits in patients with schizophrenia (Silver, Feldman et al. 2003). Specifically, the relationship between working memory and other neuropsychological functions was examined in 27 chronic male patients with schizophrenia and revealed significant correlations between verbal and spatial working memory and other neuropsychological tests (Silver, Feldman et al. 2003). Such significant relationships were not observed in healthy
subjects therefore supporting the hypothesis that working memory is a core deficit in schizophrenia.

A wealth of studies has examined working memory function in patients with schizophrenia which has produced robust and reliable reports of deficits across a range of measures (Lee and Park 2005). For example, a recent meta-analysis performed on 187 studies that examined working memory in schizophrenia compared to healthy subjects revealed deficits in all domains of working memory (Forbes, Carrick et al. 2009). First, studies that examined verbal working memory tests such as the digit span, letter-number span, and passage recall showed that patients with schizophrenia performed significantly worse compared to healthy subjects (Forbes, Carrick et al. 2009). Second, analysis on tests of visuospatial working memory including the spatial span forwards and backwards, tests of pattern recognition, and the complex figure reproductions tests demonstrated significant impairment in patients with schizophrenia compared to healthy subjects (Forbes, Carrick et al. 2009). Lastly, patients with schizophrenia were also found to perform significantly worse than healthy subjects on executive working memory tests such as self-ordered pointing task, executive golf task, and the random number/letter generation (Forbes, Carrick et al. 2009). Importantly, no relationship was found between current IQ with task performance therefore indicating that the robust finding of impaired working memory function in patients with schizophrenia was not simply attributed deficits in IQ (Forbes, Carrick et al. 2009). The advancement of neuroimaging techniques has allowed for further investigation on the nature of working memory deficits in patients with schizophrenia and will be reviewed in the following section.
1.6.2.2 Review of Neuroimaging Techniques

1.6.2.2.1 Positron Emission Tomography

PET is a three-dimensional functional imaging technique that was first introduced in the 1950s. This technique involves the labeling of radioactive chemicals injected into the bloodstream (Ter-Pogossian, Phelps et al. 1975). The system then detects pairs of gamma rays emitted by the radionuclide (tracer) that accumulates in different regions of the brain depending on the cognitive task being performed in the scanner. That is, radioligands selectively bind to specific neurotransmitter receptors that will allow the visual reconstruction of the brain areas involved and the mechanism underlying the cognitive task being tested. However, one disadvantage of PET is the potential of ionizing radiation exposure.

1.6.2.2.2 Functional MRI

Magnetic resonance imaging (MRI) was introduced in the 1970s and is a detailed imaging technique to visualize internal structures of the body. Moreover, MRI uses a powerful magnetic field that aligns the nuclear magnetization of the hydrogen atoms of water in the body. This technique is particularly useful in imaging the brain as white and gray matter contains different amount of water thereby generating different contrasts in the MRI. Seigi Ogawa later extended this method and introduced functional MRI (fMRI) in the 1990s which allows the visualization of activated neuronal tissue (Ogawa, Lee et al. 1990). Functional MRI is based on the presence of deoxyhemoglobin in blood that changes the proton signal from water molecules surrounding a blood vessel in gradient echo MRI which generates blood oxygenation level-dependent (BOLD) contrast. Functional MRI therefore allows the visualization of BOLD activation in neuronal tissue while human subjects perform cognitive tasks. Spatial resolution is the advantage of MRI over other techniques such as electroencephalogram (EEG), while temporal resolution is limited.
Moreover, activation of the BOLD response does not indicate the nature of neuronal activity (i.e., inhibitory versus excitatory).

1.6.2.2.3 Magnetoencephalography

David Cohen (1968) first introduced MEG which is a technique similar to EEG (method described in the next section) that measures the magnetic fields induced by the electrical currents that naturally occur in the brain. MEG is advantageous over EEG since the magnetic fields are not distorted by the skull and requires less preparation. MEG, however, is more costly than EEG as this technique requires a magnetically shielded room and the equipment must be kept cool by liquid helium. Current MEG machines involve a helmet-shaped dewer that contains approximately 300 sensors that is placed just above the subject’s head. Another disadvantage of MEG is that it takes approximately 50 000 active neurons to generate a signal and these signals mostly originate from tangential dipoles located in the sulci wall compared to EEG that is more sensitive to radial dipole sources (Srinivasan, Winter et al. 2006).

1.6.2.3 Neuroimaging Working Memory

A rich literature exists on the examination of working memory with neuroimaging techniques. Such studies have attempted to support the multiple theories of working memory particularly with regards to unitary versus non-unitary systems. First, monkey studies have shown through single unit recordings that increased firing in the neurons of the lateral prefrontal cortex during the delay period of working memory tasks (Fuster 1995; Goldman-Rakic 1995). Moreover, monkey lesion and neurophysiological studies suggest that the DLPFC is the brain region specialized for the storage of spatial information while the ventrolateral prefrontal cortex is involved in the storage of non-spatial information. Such division of domains have been mapped onto the conventional ‘what’ (non-spatial) and ‘where’ (spatial) visual pathways of the brain.
(Curtis and D'Esposito 2004). In line with this suggestion, monkey anatomical studies have demonstrated that the parietal region that is specialized for vision predominantly projects onto the DLPFC (Petrides and Pandya 1984; Cavada and Goldman-Rakic 1989), whereas the temporal cortex that is specialized for object vision projects more to the ventrolateral prefrontal cortex (Barbas 1988). Functional neuroimaging studies in humans, however, have failed to demonstrate this anatomical division between spatial and non-spatial information (D'Esposito, Aguirre et al. 1998). Instead, this analysis plotted all the functional imaging studies up to the year of its publication and found a hemispheric organization for spatial and non-spatial information rather than a DLPFC versus ventrolateral prefrontal cortex, respectively. That is, the findings of D’Esposito et al (1998) demonstrate that the left hemisphere is specialized for the mediation of non-spatial information whereas the right hemisphere mediates spatial information (D'Esposito, Aguirre et al. 1998).

Considering the wealth of neuroimaging studies on working memory with objectives to investigate the multiple theories of this cognitive process, this section will limit its discussion to studies that have employed the N-back task in healthy subjects as this task paradigm was employed in the experiments described in the proceeding chapters. In this regard, Owen et al (2005) conducted a meta-analysis on 24 studies that employed the N-back task testing both manipulation (location versus identity monitoring) and content (verbal versus non-verbal) variants in healthy subjects. Across studies, robust activations were found in the lateral and medial premotor cortex, dorsal cingulate, DLPFC, ventrolateral prefrontal cortex, frontal poles, and the medial and lateral posterior parietal cortex (Owen, McMillan et al. 2005). Subsidiary meta-analysis on the primary data revealed similar broad activation patterns for identity monitoring of verbal information and both location and identity monitoring of non-verbal
information. This analysis therefore reveals the involvement of a frontoparietal system in the mediation of both verbal and non-verbal working memory tasks (Owen, McMillan et al. 2005).

1.6.2.3.1 Neuroimaging Evidence for Working Memory Deficits in Schizophrenia

The nature of abnormal brain activation while performing working memory tests in fMRI remains controversial owing to the inconsistent findings in literature. That is, some studies have found evidence of enhanced activation of the DLPFC (Callicott, Bertolino et al. 2000; Manoach, Gollub et al. 2000), no difference (Honey, Bullmore et al. 2002; Walter, Wunderlich et al. 2003; Kindermann, Brown et al. 2004; Walter, Vasic et al. 2007), and reduced activity (Callicott, Ramsey et al. 1998; Barch, Carter et al. 2001; Perlstein, Carter et al. 2001; Barch, Csernansky et al. 2002) while patients with schizophrenia perform working memory tasks. It has also been suggested that reduced activation of the DLPFC is predicted by adjacent white matter disturbances shown through diffusion tensor imaging (Schlosser, Nenadic et al. 2007). Meta-analyses performed on working memory studies in patients with schizophrenia also are inconsistent with regards to the nature of DLPFC activation. For example, a recent meta-analysis of 12 studies that tested patients with schizophrenia on the N-back task revealed significantly lower activation of the DLPFC (Glahn, Ragland et al. 2005). By contrast, a more inclusive meta-analysis on 29 studies of working memory failed to show a difference in DLPFC activation in patients with schizophrenia compared to healthy subjects (Van Snellenberg, Torres et al. 2006). However, the meta-analysis conducted by van Snellenberg et al (2006) revealed a relationship across studies between DLPFC differences between patient and control groups and patient-control differences in task performance. That is, poorer performance in patients with schizophrenia was predictive of lower DLPFC activation thereby suggesting that the findings of reduced DLPFC activation may be attributed to greater performance deficits in patients (Van
Snellenberg, Torres et al. 2006). In line with this finding, Callicott et al (2003) examined DLPFC activation while performing the N-back task and compared high versus low performing subjects in both patient and control samples (Callicott, Mattay et al. 2003). When low-performing patients were compared to either low or high performing healthy subjects, reduced DLPFC was found, whereas, when high performing patients were compared to low performing healthy subjects, increased DLPFC was revealed in patients with schizophrenia (Callicott, Mattay et al. 2003).

Furthermore, when high performing patients were compared to high performing healthy subjects, discrete regions of the DLPFC were found to be more active thereby suggesting that patients with schizophrenia may employ a different brain network compared to healthy subjects in order to achieve a greater level of performance on the N-back task (Callicott, Mattay et al. 2003).

In addition to task performance, other factors such as medication or physical gray matter differences have been suggested to contribute to the inconsistent activation of the DLPFC during working memory performance. For example, several studies have reported that patients treated with second generation antipsychotic medication show increased prefrontal activation during working memory tasks (Honey, Bullmore et al. 1999; Meisenzahl, Scheuerecker et al. 2006; Wolf, Janssen et al. 2007) however this effect was not related to working memory performance. The impact of medication on DLPFC therefore remains unclear. Further, greater heterogeneity in DLPFC activation in patients with schizophrenia due to differences in the morphology of the prefrontal cortex may also result in decreased DLPFC when converted to a standard template (Manoach, Gollub et al. 2000). Finally, differences in DLPFC may also result from the domain of working memory tested (i.e., verbal versus non-verbal tests) and other task parameters (Manoach 2003).
1.6.3 Working Memory: The Role of Neurotransmitter Systems

1.6.3.1 Dopamine System

Pharmacological manipulation in both animals and humans has suggested that dopamine may enhance working memory performance. In monkeys, following 6-hydroxy-dopamine lesions in the prefrontal cortex or the administration of dopamine antagonists impair working memory function (Sawaguchi and Goldman-Rakic 1994). By contrast, the administration of low dose dopamine agonists improves working memory performance in monkeys (Williams and Goldman-Rakic 1995). Dopamine agonists have also been shown to improve working memory function in humans. For example, the administration of non-selective agents, such as amphetamine and methylphenidate, has been demonstrated to improve accuracy and reaction time in healthy subjects (Barch and Carter 2005). Furthermore, Mintzer and Griffiths (2003) observed improved performance on the 2-back condition of the N-back task (Mintzer and Griffiths 2003), although it has been suggested that this effect may be limited to poor baseline performers (Mattay, Berman et al. 1996). Finally, the administration of amphetamine has been demonstrated to ameliorate deficits in working memory in sleep deprived individuals (Pigeau, Naitoh et al. 1995; Magill, Waters et al. 2003). In general, the findings of these studies suggest that amphetamine and methylphenidate may improve working memory performance though there is some evidence that suggests that this effect may have a greater impact on those with relatively poor baseline performance.

The role of dopamine in working memory has been suggested to increase the stability of active representations in memory in order to adequately respond to task-relevant stimuli (Durstewitz, Seamans et al. 2000). Braver and colleagues (1999) have also suggested that dopamine may serve as a cue for updating information in working memory and that phasic dopamine signals may help to regulate what information is encoded in working memory. Furthermore, Braver et al
suggest that dopamine's role in working memory may be to protect against interference of distracting or competing information (Braver, Barch et al. 1999; Braver and Cohen 1999). Hazy et al (2007) has further suggested that the basal ganglia mediated by dopamine signals serve to update representations in working memory (Hazy, Frank et al. 2007).

1.6.3.1.1 Pharmacological Manipulation of Dopamine on Working Memory in Schizophrenia

There are a few studies which have examined the impact of dopamine on working memory function in patients with schizophrenia. For example, the administration of apomorphine to medication withdrawn patients with schizophrenia resulted in no significant change in performance, but a significant task-related increase in blood flow to the DLPFC was observed (Daniel, Berman et al. 1989). More recently, the administration of amphetamine in medicated patients with schizophrenia has shown some promise. For example, Barch and Carter (2005) administered amphetamine and a placebo-control to stable medicated patients with schizophrenia which was found to enhance performance on a spatial delayed match to sample task evidenced through improved accuracy and reaction time (Barch and Carter 2005). However, improved performance was observed in conditions with and without a delay period thereby suggesting that amphetamine increased the encoding subprocess of the working memory task rather than the storage of information (Barch and Carter 2005). These studies therefore provide reasonable evidence for the positive impact of amphetamine on working memory performance in medicated patients with schizophrenia through the augmentation of dopaminergic function.

1.6.3.2 Noradrenergic System

The administration of noradrenergic agonists have also been demonstrated to have a positive influence on working memory particularly in animals while less consistent findings have been found in humans. For example, a couple of studies have administered noradrenergic alpha-2
agonists in monkeys which resulted in improved working memory performance, particularly in aged animals (Arnsten, Cai et al. 1995; Arnsten and Goldman-Rakic 1998). Such improvement in working memory function with alpha-2 agonists was suggested to protect against interference thereby decreasing distractibility (Arnsten and Contant 1992). In humans, however, there has been mixed results in terms of the effect of alpha-2 agonists on working memory function. For example, Coull et al (1995) found that alpha-2 agonist clonidine improved spatial self-ordered pointing in healthy subjects but this effect was only observed if subjects were well-practiced and with 2.5 µg/kg, while administration of 1.5 µg/kg doses impaired performance (Coull, Middleton et al. 1995). Other studies by Jakala et al (1999a, 1999b) demonstrated that clonidine at both low (0.5, 2 µg/kg) and higher doses (5 µg/kg) impaired working memory performance on the spatial delayed match to sample and the self-ordered pointing tasks evident through an increased number of errors or by the increase in reaction time (Jakala, Riekkinen et al. 1999; Jakala, Sirvio et al. 1999). The current literature on the impact of alpha-2 agonists on working memory performance is positive in non-human primates however this effect has been inconsistent with human subjects. If patients with schizophrenia have impaired noradrenergic function then it is possible that alpha-2 agonists may have a positive impact on working memory performance in this disorder.

1.6.3.3 Acetylcholine System

The influence of the cholinergic agents on working memory has also been examined. For example, Furey et al (1997) demonstrated that the cholinesterase inhibitor phystostigmine improved performance on the face delayed to match sample task (Furey et al 1997) that may be related to a reduction of task-related prefrontal activity and increased activity in the extrastriate brain regions (Furey, Pietrini et al. 2000; Furey, Pietrini et al. 2000). The authors suggested that increased activity in the visual cortex may have improved the encoding of visual information,
which could have contributed to improved working memory performance (Furey, Pietrini et al. 2000). The studies conducted by Furey and colleagues suggest that cholinergic agents may have a positive impact on working memory in patients with schizophrenia but more studies are needed.

1.6.3.4 Serotonin System

The literature on the role of the serotonin system in working memory is limited. Luciana et al (1998) administered a serotonin agonist fenfluramine to human subjects and observed a decrease in delayed spatial memory (Luciana, Collins et al. 1998). A later study by this group administered tryptophan which is a precursor to serotonin and demonstrated impairment in performance on the digit span backwards and affective working memory (Luciana, Burgund et al. 2001). Together these two studies suggest that enhancing serotonin activity would not have a positive benefit on working memory in human subjects.

1.6.3.5 GABAergic System

Although working memory is considered an emergent property of a neuronal network that involves a number of different brain regions (Goldman-Rakic 1988), it is reliant on the coordinated and sustained firing of neurons within the DLPFC to mediate the temporary presentation of a stimulus cue and the initiation of the behavioural response in working memory (Goldman-Rakic 1995). As reviewed in a previous section, DLPFC neurons use GABA as the principle neurotransmitter and thus the role of GABA in working memory has been extensively studied.

Inhibitory interneurons that use GABA are believed to provide the mechanism in which to synchronize pyramidal neuronal activity during working memory processes (Lewis, Hashimoto et al. 2005). This proposal has been supported by several studies in monkeys. For example,
during the delay period of working memory tasks, Wilson et al (1994) demonstrated sustained activity in the DLPFC of monkeys (Wilson, O'Scalaidhe et al. 1994) that has been suggested to be important in both the task-related neuronal firing and the spatial tuning of neuronal responses during working memory (Rao, Williams et al. 2000). In line with this suggestion, Sawaguchi et al (1989) injected GABA antagonist bicuculline into the DLPFC of monkeys and observed a disruption in working memory performance (Sawaguchi, Matsumura et al. 1989). However, decrements in working memory performance following the administration of baclofen, a GABA<sub>B</sub> receptor agonist has been shown in rats (Nakagawa and Takashima 1997; Romanides, Duffy et al. 1999). By contrast, in humans baclofen was shown to enhance working memory performance (Escher and Mittleman 2004).

1.6.3.5.1 Pharmacological Manipulation of GABA on Working Memory in Schizophrenia

Pharmacological manipulations of GABA<sub>A</sub> receptors have been examined for its effect on working memory in patients with schizophrenia compared to healthy subjects (Menzies, Ooi et al. 2007). In this study, subjects received either GABA<sub>A</sub> agonist (lorazepam), GABA<sub>A</sub> antagonist (flumazenil) or a placebo prior to the performing the N-back task (Menzies, Ooi et al. 2007). This study found that lorazepam impaired performance while flumazenil enhanced it and this effect was most pronounced in patients with schizophrenia compared to healthy subjects. Taken together, these studies suggest that inhibitory activity of the DLPFC mediated through GABA has a spatial tuning and temporal role that is critical to working memory (Constantinidis, Williams et al. 2002) and that pharmacological manipulations of GABA may improve performance in patients with schizophrenia.
Cortical Inhibition

Cortical inhibition refers to a neurophysiological mechanism in which inhibitory neurons selectively attenuate the activity of other neurons in the cortex. Pyramidal cells comprise approximately 70-80% of the cortex while interneurons make up about 15-30% (DeFelipe and Farinas 1992). Interneurons are predominantly mediated by the inhibitory neurotransmitter GABA and are classified according to the type of synapse they form (Benes and Berretta 2001). The most common cortical interneurons are basket cells which form axo-somatic inhibitory synapses with pyramidal cells around the surface of the pyramidal cell body (Benes and Berretta 2001). Moreover, basket cells are located in layers III-IV and have long axons that ascend to more superficial layers of the cortex and are also modulated by subcortical activity through direct thalamic inputs (Benes and Berretta 2001). Chandelier cells make up the second type of interneurons that form axo-axonal inhibitory synapses with the initial segment of pyramidal cells axons (Somogyi, Tamas et al. 1998; Lewis, Hashimoto et al. 2005). This configuration has been suggested to allow chandelier inhibitory interneurons to have a strong influence over pyramidal cell output (Lewis, Hashimoto et al. 2005) possibly through the modulation of the responsivity of other cortical neurons (Benes and Berretta 2001). Double bouquet cells make up the third classification of interneurons that form an axo-dendritic inhibitory synapse on the apical and basal dendrites of pyramidal cells in addition to adjacent interneurons (Somogyi and Cowey 1981). Further, double bouquet interneurons may be involved in the disinhibition of pyramidal cells to attenuate excessive inhibitory synaptic control (Somogyi, Tamas et al. 1998; Benes and Berretta 2001). Basket and Chandelier interneurons are located in cortical layers III-IV and are fast-spiking, while double bouquet interneurons are found in cortical layer II and III (Benes and Berretta 2001).
Cortical excitation reflects the balance between excitatory and inhibitory activity. Moreover, pyramidal cell activity results from both excitatory post-synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs) that terminate throughout the cell (Krnjevic 1997). This coordinated interaction of EPSPs and IPSPs is universally accepted as an important element in the control of neuronal firing. Cortical inhibition therefore reflects the activity of IPSPs generated by GABAergic interneurons and plays several important functions in the cortex including working memory (Lewis, Pierri et al. 1999; Constantinidis, Williams et al. 2002). Specific subclasses of GABA inhibitory interneurons in the DLPFC may play a critical role in regulating the activity of layer III pyramidal neurons that have been demonstrated to be important in working memory (Fuster, Bauer et al. 1985; Friedman and Goldman-Rakic 1994). For example, the spatial arrangement of the axon terminals of basket cells in which a large portion of their terminals synapse on the soma of pyramidal cells may provide a reasonable mechanism to inhibit the activity of pyramidal neurons located in the gaps between the interconnected stripes (Pucak, Levitt et al. 1996; Melchitzky, Sesack et al. 1998). Chandelier cells may also provide inhibition of layer III pyramidal neurons through the formation of vertical arrays that provide inhibitory input exclusively to the axon initial segment of pyramidal cells (Peters 1984). In addition, the spread of chandelier cell’s axon arbors is consistent with the width of the stripes formed by later III pyramidal cells further suggesting that these cells may be important in the regulation of pyramidal cell output (Lund and Lewis 1993). In the following section, the role of GABA in cortical oscillations will be discussed followed by the evidence for oscillatory activity underlying working memory function.
1.6.3.6  Neurophysiological Assessment of Working Memory

1.6.3.6.1  Electroencephalography

In the 1920s Hans Berger recorded brain waves from the human scalp and named this technique EEG. EEG is one of the most widely used techniques in which to record brain activity due to its high temporal resolution and low cost. EEG involves the placement of electrodes on the scalp and therefore mostly captures the synaptic activity from post synaptic potentials of neurons in the superficial layers of the brain (Buzsaki, 2006). Since the electric potential from a single neuron is too small to be captured by EEG, the activity recorded reflects the summation of the synchronous activity from thousands of neurons with a similar spatial orientation. Furthermore, during neuronal processing, EEG captures a finite number of discrete frequency bands that are conventionally divided into delta (1-4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (12-28 Hz), and gamma (30-50 Hz) oscillatory activity (Buzsaki 2006).

1.6.3.7  Cortical Oscillations

Over the past two decades, there has been a shift from examining event related potentials to brain oscillations following the discovery that cortical networks have a natural tendency to engage in oscillatory activity at multiple frequencies (Singer 1999; Buzsaki and Draguhn 2004; Fries, Nikolic et al. 2007). Oscillations measured by both in vitro (i.e., brain slices and patch-clamp recording) and in vivo through scalp recordings with EEG and MEG have been associated activity within different frequency bands to different states of the brain. For example, lower oscillatory activities including delta, theta, and alpha are associated with different stages of sleep, while higher frequencies have been associated with the waking brain (Buzsaki 2006).

The mechanisms underlying the generation of cortical oscillations are currently being investigated. At the single neuronal level, the gating of ion channels in the cell membrane has
been suggested to provide neurons with intrinsic properties that allow them to oscillate at different frequencies (Buzsaki 2006). That is, the open or closed state of these membrane channels is under the control of the membrane voltage, neurotransmitters and modulators, in addition to other cell factors that determine how eagerly and precisely the neuron responds to a given input. In addition to voltage gated channels, ligand-, ion-, and second messenger gated channels endow neurons with the intrinsic properties capable of generating a repertoire of activities including oscillation and resonance at multiple frequency ranges (Buzsaki 2006). Furthermore, several different ion compositions exist across various neuronal cell types which allows for cortical neurons to have a wide range of preferred frequencies, spike timing properties, and their diverse frequency-tuning properties are critical for setting network dynamics (Buzsaki 2006). For example, GABAergic interneurons respond with the highest temporal precision to frequencies in the gamma band, while pyramidal neurons respond more precisely to activities in the lower frequency ranges (Gupta, Wang et al. 2000; Thomson 2000; Markram, Toledo-Rodriguez et al. 2004).

1.6.3.7.1 Cortical Oscillations and Information Encoding

Interesting relationships have been uncovered between frequency bands that may be important in information encoding and functional roles in the cortex. For example, the power of a cortical oscillation has been shown to decrease as the frequency of the oscillation increases. Such a relationship suggests that a temporal correlation exists between frequency bands therefore oscillatory activity at higher frequencies are vulnerable to alterations at lower frequency bands (Buzsaki 2006). Furthermore, an inverse relationship has been shown between the size of the neuronal pool and the frequency of oscillatory activity. Thus, a small number of neurons tend to participate in higher frequency oscillatory activity whereas a large number of neurons are needed to participate in slower frequencies (Buzsaki 2006). In addition, activities between frequency
bands have been shown to interact with each other which may be important in information processing. For example, frequency concatenation occurs when two local networks that oscillate at distinct frequency bands are co-activated and interact to generate activity in a new frequency with a period that is the sum of the two networks (Roopun, Kramer et al. 2008). Another example is cross-frequency interaction where the power of discrete frequency band is modified by the phase of a lower frequency band that co-exists during information processing (Roopun, Kramer et al. 2008).

Information processing has also been linked to the modulation of cortical oscillations (Fries, Nikolic et al. 2007). That is, Fries et al (2007) suggested that the modulation of oscillatory responses of neurons may provide the temporal frame that determines which neurons communicate with each other (Fries, Nikolic et al. 2007). The resulting neuronal responses have further been suggested to convey information in two different orthogonal messages in parallel (Singer 1999). First, neurons should indicate the presence of the feature to which they are tuned; and second, neurons should indicate with which other neurons they are communicating with (Singer 1999). The first message is encoded in the discharge frequency of the neurons and it is suggested that the second message is contained in the precise timing relationships between individual spikes of distributed neurons thus providing the temporal code (Singer 1999). Singer further proposed that the precise timing relations are achieved either by internal timing mechanisms or by the timing of external events (stimulus locking). Internal timing mechanisms involve an oscillatory modulation of neuronal responses across different frequency bands ranging from delta to gamma ripples. These oscillations therefore limit the communication of cells to short temporal windows and the duration of these windows decreases with oscillation frequency (Singer 1999). Through the varying phase relationship between oscillating groups, networks of functionally cooperating neurons can be flexibly configured within hard wired
networks. Furthermore, the synchronization of spike emitted by neuronal population achieves greater saliency of their responses and is enhanced due to the coincidence sensitivity of receiving neurons by increasing the discharge rate (Singer 1999). Such a temporal code through the modulation of oscillatory activity may underlie the association between working memory and oscillatory activity.

1.6.3.7.2 Cortical Oscillations Provides the Temporal Framework for Working Memory

Temporal segmentation by phase encoding has been suggested to be involved in the maintenance of multiple working memory items (Jensen 2006). For example, if 5 elements A through to E are to be maintained in a working memory buffer through the repeated rehearsal of the list A-E. This mechanism involves a fast rate at which the individual items are activated and a slow rate at which the full list is repeated. Although this scheme was first proposed as a mechanism underlying short term memory (Horn and Usher 1992), Lisman and Idiart (1995) suggested that the two activation rates are associated with brain oscillations particularly in the theta and gamma frequency ranges. Furthermore, Lisman and Idiart suggested that the number of gamma cycles per theta cycle determines the working memory capacity of the buffer and that the gamma frequency determines how fast items can be retrieved (Lisman and Idiart 1995). The principle of dual oscillations can therefore account for how multiple items are maintained and retrieved in working memory (Jensen 2006).

1.6.3.8 Cortical Oscillations Association with Working Memory

Evoked and induced oscillatory activity has been associated with performing both verbal and non-verbal working memory tasks. Several studies have shown activations in the theta oscillatory activity during working memory tasks in healthy subjects (Sarnthein, Petsche et al. 1998; Klimesch, Doppelmayr et al. 2001; Raghavachari, Kahana et al. 2001; Jensen and Tesche...
2002; Schack, Vath et al. 2002). Fewer studies report activations in the alpha (Gevins, Smith et al. 1997; Klimesch, Doppelmayr et al. 1997; Jensen, Gelfand et al. 2002; Rizzuto, Madsen et al. 2003) and beta (Tallon-Baudry and Bertrand 1999; von Stein, Rappelsberger et al. 1999; Schack, Vath et al. 2002) frequency bands while healthy subjects perform working memory tasks. Finally, activations in the gamma frequency range have been consistently associated with working memory in healthy subjects (Sarnthein, Petsche et al. 1998; Tallon-Baudry and Bertrand 1999; Tallon-Baudry, Bertrand et al. 2001; Gruber and Muller 2005; Kaiser and Lutzenberger 2005).

1.6.3.8.1 Gamma Oscillations and Working Memory

Activations in the gamma frequency band have been correlated with specific working memory processes. For example, Howard et al (2003) tested epileptic patients who were undergoing intracranial EEG recordings prior to surgical resection in the classic Sternberg task and observed increases in gamma power in response to increases in working memory load (Howard, Rizzuto et al. 2003). Such a relationship between gamma power and working memory load has also been demonstrated in healthy subjects using both EEG and MEG (Tallon-Baudry, Bertrand et al. 1998; Mainy, Kahane et al. 2007; Meltzer, Zaveri et al. 2008). Increases in gamma power in response to working memory load have also been reported during non-verbal working memory tasks (Lutzenberger, Pulvermuller et al. 1995; Tallon-Baudry, Bertrand et al. 1998; Lutzenberger, Ripper et al. 2002; Pesaran, Pezaris et al. 2002). Gamma oscillatory activity has been suggested to organize and temporally segment the representations of both verbal and non-verbal items in a multi-unit working memory system (Lisman and Idiart 1995).

Gamma band activity during working memory tasks is observed in distributed network (Mainy, Kahane et al. 2007) and includes focal centres in the prefrontal cortex (BA 9, 10, 44, 45, 46), the
precentral and postcentral gyri, the auditory cortex, the fusiform gyrus, and the hippocampus which is consistent with human lesion (Petrides and Milner 1982; Frisk and Milner 1990; Owen and Craddock 1996), and neuroimaging (Jonides, Smith et al. 1993; Braver, Cohen et al. 1997; Cohen, Perlstein et al. 1997; Courtney, Ungerleider et al. 1997; Courtney, Petit et al. 1998; Martinkauppi, Rama et al. 2000; Romanski 2004) studies. Furthermore, neuroimaging studies have demonstrated that the BOLD signal in functional MRI is frequency dependent. That is, BOLD responses were found to be positively correlated at higher frequencies including the gamma frequency range, while activity within the lower frequency ranges (i.e., alpha power) was correlated in a negative fashion (Logothetis, Pauls et al. 2001; Foucher, Otzenberger et al. 2003; Moosmann, Ritter et al. 2003; Brookes, Gibson et al. 2005; Mukamel, Gelbard et al. 2005; Niessing, Ebisch et al. 2005). These studies together suggest that activations within the gamma frequency band are involved in specific working memory subprocesses and are robustly observed in the DLPFC that is critical to this cognitive process.

1.6.3.8.2 The Generation and Modulation of Gamma Oscillations
GABAergic receptor mediated inhibitory neurotransmission plays a critical role in generation and modulation of gamma oscillations. There are several studies which suggest that GABA_{A} receptor mediated IPSPs contribute to the generation of gamma oscillations (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007). For example, GABA_{A} receptors typically discharge at a rate of 30-50 Hz (i.e., gamma frequency band) which induces a high-frequency on-off oscillatory pattern of pyramidal cell discharge when recorded by EEG. In the rat, Whittington et al (1995) demonstrated that the activation of glutamate receptors in hippocampal and neocortical slices resulted in synchronous 40 Hz oscillatory activity in the networks of inhibitory neurons that are connected by synapses comprising GABA_{A} receptors. Whittington et al (1995) further demonstrated that the administration of a GABA_{A} antagonist
bicuculline resulted in the amelioration of these gamma oscillations (Whittington, Traub et al. 1995). In addition, Wang and Buzsaki showed through computer simulations that the synaptic time constant for GABA_A receptors approximately ranges from 10 to 25 milliseconds further implicating GABA_A receptor activity in the generation of gamma oscillations (Wang and Buzsaki 1996). In line with this finding, the prolongation of the decay period of the GABA_A receptor post synaptic current with barbiturate was shown to reduce gamma oscillations (Fisahn, Pike et al. 1998).

The modulation of gamma oscillations have been suggested to be achieved through GABA_B receptor mediated IPSPs (Whittington, Traub et al. 1995; Brown, Davies et al. 2007; Leung and Shen 2007). In this regard, in vivo and in vitro studies have demonstrated that the activation of GABA_B receptors results in the suppression of both spontaneous and stimulus induced gamma oscillatory activity. For example, the administration of baclofen, a GABA_B agonist was shown to ameliorate gamma oscillations in rat hippocampal slices (Brown, Davies et al. 2007), while the blockade of GABA_B receptors in the hippocampus of behaving rats results in an increase in gamma oscillations (Leung and Shen 2007). Second, the reduction of parvalbumin has been shown to result in increased facilitation of GABA release during repetitive synaptic firing has been directly linked gamma modulation (Vreugdenhil, Jefferys et al. 2003; Sohal, Zhang et al. 2009). Finally, through transcranial magnetic stimulation (TMS) studies the activity of GABA_B receptors has been shown to have an inhibitory effect on GABA_A receptor activity (Sanger, Garg et al. 2001; Daskalakis, Christensen et al. 2002) with an inhibitory effect that typically ranges from 250 to 500 milliseconds (McCormick 1989).
1.6.3.9 Analyzing Cortical Oscillations

Oscillatory activity recorded during working memory can be quantified by two different EEG analytical techniques. The first method examines evoked oscillatory responses that are phase-locked to the stimulus onset with a fixed latency within the first 100 msec following stimulus onset and are measured by stimulus-triggered averaging of responses (Tallon-Baudry, Kreiter et al. 1999). The second method involves the examination of induced oscillatory activity that is not phase-locked to stimulus onset and jitters in latency varying from trial to trial; thus, responses are cancelled out when averaged (Tallon-Baudry, Kreiter et al. 1999). Although both evoked and induced oscillatory activities have been associated with working memory (Howard, Rizzuto et al. 2003; Basar-Eroglu, Brand et al. 2007), induced oscillatory activity in the gamma frequency band has been suggested to reflect microsaccadic eye movement rather than neuronal processing during cognitive paradigms (Yuval-Greenberg, Tomer et al. 2008). That is, when Yuval-Greenberg et al (2008) recorded EEG simultaneously with high resolution binocular eye tracking demonstrated that increased gamma power reflected in the single-trial EEG response is in fact transient in nature rather than an index of sustained neuronal oscillatory activity. Second, the observed transient increase in gamma power was time locked to- and coincided with the onset of miniature saccades, while the time course reflected the dynamics of these saccadic eye movements. These findings therefore suggest that induced gamma band activity is a direct consequence of miniature saccades rather than neuronal oscillations in the gamma frequency range. Yuval-Greenberg et al (2008), therefore, contend that the evaluation of evoked rather than induced gamma oscillatory activity mitigates the effect of miniature saccades on this neurophysiological phenomenon (Yuval-Greenberg, Tomer et al. 2008). Additionally, gamma band activity during cognitive tasks has been shown to reflect cranial musculature artifact (Shackman 2010), however, since this activity is characterized by irregular spikes and waves
present in all spectral frequencies such artifact should be significantly reduced when multiple trials are averaged with evoked analytical methods (Barr and Daskalakis 2010).

1.6.4 Neurophysiological Evidence for Working Memory Deficits in Schizophrenia

Oscillatory activity during working memory performance has started to be examined in patients with schizophrenia. Recent studies have observed alterations in oscillatory activities across spectral frequencies in patients with schizophrenia compared to healthy subjects.

1.6.4.1 Delta

Spectro-temporo-spatial MEG patterns have been examined recently by Ince et al (2009) while patients with schizophrenia medicated with non-conventional antipsychotics and healthy subjects while performing the Sternberg task. With this technique, changes in oscillatory amplitude are captured. That is, when an event causes an amplitude decrease in rhythmic activity this is referred to as event-related desynchronization (ERD), while increases in amplitude are referred to as event-related synchronization (ERS) (Pfurtscheller and Lopes da Silva 1999; Neuper and Pfurtscheller 2001). Furthermore, these oscillatory power patterns have been shown to reflect increased cortical excitability with ERD and decreased or inhibited cortical excitability with ERS (Neuper and Pfurtscheller 2001). This study revealed alterations in delta ERD/ERS patterns in the dorso-frontal, occipital and left fronto-temporal brain regions in patients with schizophrenia during the encoding stage of working memory (Ince, Pellizzer et al. 2009). Stephane et al (2008) also have examined ERD/ERS patterns during verbal working memory in 10 patients with schizophrenia on nonconventional antipsychotic medication compared to 11 healthy subjects. Using the Sternberg task, reduced ERD/ERS patterns were found in patients with schizophrenia compared to healthy subjects during the encoding and maintenance stages of working memory.
Furthermore, patients with schizophrenia showed a lack of delta activity in the left frontal and left parietal brain regions compared to healthy subjects (Stephane, Ince et al. 2008).

1.6.4.2 Theta

In 2005, Schiemdt et al examined event related (induced) theta oscillatory during the N-back task combined with a task switching task in 10 medicated in-patients diagnosed with schizophrenia compared to 10 healthy subjects (Schmiedt, Brand et al. 2005). The N-back task was administered at 3 different working memory loads: 0-2 back with the added component of response instructions (i.e., which hand to respond with left or right) while EEG was recorded. Schmiedt et al (2005) reported increased theta oscillatory activity with increased working memory load and rule-switching in the fronto-central region in healthy subjects. Moreover, such modulation of theta oscillatory activity with working memory and task-related increases was not observed in any brain region in patients with schizophrenia (Schmiedt, Brand et al. 2005).

Alterations in theta have also been reported by Haenschel et al (2009) who examined both induced and evoked oscillatory activities in 14 early-onset patients with schizophrenia compared to healthy subjects (Haenschel, Bittner et al. 2009). In their study, a delayed discrimination task was used to examine oscillatory activity during the encoding, maintenance and retrieval stages of visual working memory at 3 different loads. Haenschel et al (2009) observed decreased evoked theta oscillatory activity during the encoding stage and a decrease in induced theta activity during the retrieval stage in patients with schizophrenia compared to healthy subjects (Haenschel, Bittner et al. 2009).

1.6.4.3 Alpha

Bachman et al (2008) were the first to examine working memory in 29 pairs of discordant twins with schizophrenia (Bachman, Kim et al. 2008). In this study, spatial working memory was
tested at 4 different working memory loads to examine ERD/ERS patterns with MEG. Similar ERD/ERS patterns over the posterior brain region at low working memory loads was observed; however with increased working memory loads, patients and to an intermediate degree their non-schizophrenic co-twins (both monozygotic and dizygotic pairs collapsed together) demonstrated significantly greater increases in ERD/ERS patterns in the alpha frequency band compared to healthy subjects (Bachman, Kim et al. 2008). Alterations in alpha oscillatory activity have also been reported in the previously described studies by Ince et al (2009) and Stephane et al (2008). By contrast, no differences were reported by Haenschel et al (2009) in induced alpha oscillatory activity during the maintenance stage in patients with schizophrenia compared to healthy subjects.

1.6.4.4 Beta
There are few reports of altered beta oscillatory activity in patients with schizophrenia during working memory. In the study described previously, Stephane et al (2008) also reported an absence of ERD/ERS beta activation in the left frontal and parietal lobes in patients with schizophrenia compared to healthy subjects while performing the Sternberg task (Stephane, Ince et al. 2008). In addition, decreased evoked beta oscillatory activity was observed in patients with schizophrenia during the encoding stage of visual working memory compared to healthy subjects (Haenschel, Bittner et al. 2009).

1.6.4.5 Gamma
Examination of gamma oscillatory activity in patients with schizophrenia during working memory has yielded inconsistent findings. For example, studies have reported both increased and decreased and no differences in gamma oscillatory activity in patients with schizophrenia compared to healthy subjects. For example, Basar-Eroglu (2007) administered the N-back at 3
working memory loads (0-back, 1-back and 2-back) in 10 medicated in-patients with schizophrenia compared to 10 healthy subjects and reported an increase in evoked gamma oscillatory activity with working memory load in healthy subjects, while patients exhibited increased gamma activity at each working memory load in the frontal brain region (Basar-Eroglu, Brand et al. 2007). That is, regardless of task difficulty, patients elicited increased gamma oscillatory activity even during the 0-back without any memory component. By contrast, Lewis and colleagues (Cho, Konecky et al. 2006; Lewis, Cho et al. 2008) reported decreased induced gamma oscillatory activity in the frontal region of 16 medicated patients with schizophrenia compared to healthy subjects. In addition, these studies reported a lack of gamma modulation with increased working memory load consistent with the study by Basar-Eroglu (2007). More recently, Haenschel et al. (2009) found no differences in either induced or evoked gamma oscillatory during the maintenance stage of visual working memory. However, during the retrieval stage, reduced induced gamma oscillatory activity was observed in patients with schizophrenia compared to healthy subjects (Haenschel, Bittner et al. 2009).

1.6.4.6 Relationship with Behavioural Performance and Clinical Symptoms

Although the studies reviewed above demonstrate alterations in both the generation and modulation of oscillatory activity while performing working memory tasks, these studies failed to find a relationship between behavioural performance and oscillatory activity. Further, no relationship has been uncovered between oscillatory activity and the severity of clinical symptoms. Relationships have been uncovered between gamma oscillatory activity with both positive and negative symptoms although through an auditory odd-ball task and not working memory (Lee, Williams et al. 2003). In this study, patients were divided by symptom
predominance and found that positive symptoms correlated with increased gamma, while negative symptoms were related to a reduction of gamma (Lee, Williams et al. 2003).

1.6.4.7 Summary
The lack of gamma modulation with working memory load in schizophrenia patients may be attributed to alterations in GABA interneurons in the DLPFC (Akbarian, Kim et al. 1995; Benes and Berretta 2001; Lewis, Hashimoto et al. 2005; Hashimoto, Bazmi et al. 2008) critical to gamma oscillatory activity. Considering the importance of GABA in the generation (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007) and modulation (Whittington, Traub et al. 1995; Brown, Davies et al. 2007) of gamma oscillations, GABAergic impairments in patients with schizophrenia may underlie altered modulation of gamma oscillatory activity while performing working memory tasks.

1.6.5 Treatment for Working Memory Deficits in Schizophrenia
1.6.5.1 Non-Pharmacological
Non-pharmacological treatments such as cognitive remediation programs that include drill and practice exercises, teaching strategies to improve cognitive functioning, as well as, compensatory strategies and group discussions have shown modest improvements on cognition, but with no effects on working memory (McGurk, Twamley et al. 2007; Dickinson, Tenhula et al. 2010). Other computer-based programs that focused on the remediation of verbal working memory in schizophrenia through auditory training exercises have also shown promise (Adcock, Dale et al. 2009; Fisher, Holland et al. 2009). For instance, Fisher et al (2009) demonstrated significant improvements on the letter number span working memory task in patients with schizophrenia following 50 hours of auditory training exercises compared to a control group. Furthermore, this
training also improved auditory psychophysical performance that was found to be related to improved verbal working memory and global cognition (Fisher, Holland et al. 2009).

1.6.5.2 Pharmacological

Pharmacological studies have also examined differences in antipsychotic medications on cognitive functioning in patients with schizophrenia. While showing small effects toward improved cognitive performance with treatment, some studies show therapeutic advantages of second generation antipsychotics compared to conventional antipsychotics (Woodward, Purdon et al. 2005) while others have not (Keefe, Bilder et al. 2007). Even clozapine, which is the prototypic second generation agent for treatment resistant schizophrenia, was not shown to be superior to other second generation antipsychotics suggesting that medications only result in marginal therapeutic effects in schizophrenia (Harvey, Sacchetti et al. 2008). Several pharmacological adjunct treatments have been shown to be of some efficacy for cognitive dysfunction in schizophrenia. For example, the administration of galantamine, a combined acetylcholinesterase inhibitor and allosteric potentiator of the nicotinic receptor, led to modest improvements in attention (Bora, Veznedaroglu et al. 2005), delayed memory (Schubert, Young et al. 2006), recognition (Lee, Lee et al. 2007), as well as colour naming on the Stroop test (Lee, Lee et al. 2007) without any improvements in working memory. Barch and Carter (2005) conducted a double-blind placebo-controlled study that examined the effects of amphetamine (D-AMPH), a dopaminergic agonist, on cognition in schizophrenia and reported a decrease in reaction time on spatial working memory and the Stroop task in addition to improved accuracy across delay periods on the spatial working memory task (Barch and Carter 2005). Finally, recombinant human erythropoietin is a neuroprotective agent that has also been examined for its effects on cognition in chronic schizophrenia patients. In a double-blind, placebo-controlled study, significant improvements on the Repeatable Battery for the Assessment of
Neuropsychological Status language-semantic fluency, attention-coding, delayed memory recall, and working memory subtest attention-digit span compared to baseline measures following 12 weeks of recombinant human erythropoietin administration. These studies demonstrate that a number of behavioural and pharmacological approaches have been investigated as possible treatments for cognitive dysfunction in patients with schizophrenia. Although these studies show some promise, it is clear that more efficacious treatments for cognitive deficits in schizophrenia are still needed.

1.7 Repetitive Transcranial Magnetic Stimulation

Since Barker et al (1985) first introduced TMS as a non-invasive tool for the investigation of motor cortex, repetitive applications of this technique has since been employed to study the influence on a variety of cerebral functions. TMS involves the placement of an electromagnetic coil over the scalp to produce an intense and localized magnetic field which can result in either excitatory or inhibitory activation. Repetitive TMS (rTMS) uses alternating magnetic fields applied at the same frequency to induce electric currents in the cortical tissue (Burt, Lisanby et al. 2002). Low-frequency (≤ 1 Hz) rTMS is believed to cause inhibition of neuronal firing in a localized area and is used to induce virtual lesions to examine a brain region’s role in different tasks. High-frequency rTMS (>1 Hz) is believed to be excitatory in nature and can result in neuronal depolarization under the stimulating coil (Haraldsson, Ferrarelli et al. 2004). Moreover, the effect of rTMS is not limited to the targeted brain region as changes can also occur at distant interconnected sites of the brain. The application of rTMS over brain regions such as the DLPFC implicated in the pathophysiology of neuropsychiatric disorders including schizophrenia therefore represents a possible mechanism in which to influence subcortical regions that regulate
emotion and behaviour (Conca, Koppi et al. 1996; Ben-Shachar, Belmaker et al. 1997; Post and Keck 2001; Burt, Lisanby et al. 2002; Gershon, Dannon et al. 2003).

1.7.1  Induced Cortical Changes with rTMS

Cortical changes induced by rTMS have been shown in both animal models and in humans. In the rat brain, rTMS has been shown to induce significant changes in neuronal circuits evidenced by changes in behaviour and an attenuation of the hypothalamic-pituitary-adrenocortical system (Fleischmann, Prolov et al. 1995; Keck, Welt et al. 2002). Furthermore, it has been demonstrated that rTMS increases dopamine in the dorsal hippocampus (Keck, Welt et al. 2002) and the nucleus accumbens (Keck, Welt et al. 2002; Erhardt, Sillaber et al. 2004) using microdialysis in rodents. Repetitive TMS in the rat has also been shown to change in the expression of proteins reflected by the synthesis of GABA by two isoforms of GAD (Trippe, Mix et al. 2009). That is, Trippe et al (2009) demonstrated that 1 Hz rTMS in the rat reduced expression of GAD67 and increased the expression of GAD65 and GABA transporter (GAT-1) compared to sham stimulation (Trippe, Mix et al. 2009). In humans, rTMS has been shown to induce changes in cortical inhibition. For example, increased rTMS frequency up until 20 Hz applied to the motor cortex has been shown to enhance indexes of GABA\textsubscript{B} receptor mediated inhibitory neurotransmission (Daskalakis, Moller et al. 2006) but with 25 Hz stimulation a reduction in this activity has also been shown (Khedr, Rothwell et al. 2007). Repetitive TMS has also been shown to induce changes in indexes of GABA\textsubscript{A} receptor mediated inhibitory neurotransmission applied at 5 Hz over the motor cortex (Takano, Drzezga et al. 2004) and following 10 Hz (Jung, Shin et al. 2008). In addition, combined rTMS/PET studies in healthy subjects demonstrated that 10 Hz rTMS over the DLPFC resulted in increased levels of extracellular dopamine (Strafella, Paus et al. 2001), while 1 Hz rTMS resulted in increased regional blood flow in the stimulation site (DLPFC) and in the ventrolateral prefrontal cortex (Eisenegger, Treyer et al. 2008). Taken
together, animal and human studies demonstrate that rTMS has the potential to change cortical excitability through modulation of different neurotransmitters including dopamine and GABA.

1.7.2 Evidence for Working Memory Improvement in Healthy Subjects with rTMS

In healthy subjects, rTMS has been employed to investigate the roles of different brain regions in working memory by inducing virtual lesions (For example, (Sandrini, Rossini et al. 2008)) and also to examine if rTMS improves performance (Luber, Kinnunen et al. 2007; Hamidi, Tononi et al. 2008; Hamidi, Tononi et al. 2009; Postle and Feredoes 2009; Preston, Anderson et al. 2009). Specifically, high frequency rTMS has been shown to reduce reaction times while performing working memory tasks when applied to the parietal cortex (Luber, Kinnunen et al. 2007), superior parietal lobule (Hamidi, Tononi et al. 2008) and to the DLPFC (Preston, Anderson et al. 2009). Improvements in working memory performance accuracy have also been reported. For example, Feredoes and Postle (2009) applied 8 Hz rTMS over the left inferior frontal gyrus while healthy subjects performed a working memory task (response deadline task) and observed an improvement in the false alarm rate in response to interference probes (Postle and Feredoes 2009). Hamidi et al (2009) examined the effect of rTMS applied over the superior parietal lobule and the DLPFC on delayed recognition and recall working memory tasks in healthy subjects. This study reported that only 10 Hz rTMS over the right DLPFC resulted in an increase in accuracy on the delayed recognition task with no differences found with superior parietal lobule application (Hamidi, Tononi et al. 2009). Although there are a limited number of studies that have demonstrated an improvement in performance with rTMS, it is possible that rTMS may have a greater effect in patients with schizophrenia with severe deficits in working memory function. In addition, the application of repeated sessions of rTMS may be more effective in
enhancing working memory performance in both healthy subjects and in patients with schizophrenia.

1.7.3 Repetitive TMS as a Treatment for Schizophrenia

Recently, rTMS has been examined as treatment options for patients with schizophrenia owing to the growing observation of non-responders to antipsychotic medication (Hajak, Marienhagen et al. 2004; Jansma, Ramsey et al. 2004; Hoffman, Gueorguieva et al. 2005). In addition, electroconvulsive therapy is proven for its anti-depressant effects however it tends to substantiate cognitive impairments in patients with schizophrenia (Squire 1982; Sackeim, Portnoy et al. 1986; Weiner, Rogers et al. 1986). In this regard, rTMS has been examined for its treatment effects on auditory hallucinations, negative symptoms (Hoffman, Boutros et al. 2000; Hoffman, Hawkins et al. 2003; Hoffman, Gueorguieva et al. 2005; Jandl, Bittner et al. 2005; Poulet, Brunelin et al. 2005) and more recently on cognitive deficits in this disorder. For example, seminal studies conducted by Hoffman and colleagues (2000, 2003) applied 1 Hz rTMS over the left temporoparietal cortex of 24 patients over the course of nine days. In 9 of 12 patients who received active stimulation experienced a greater than 50% reduction in the frequency and severity of their auditory hallucinations as compared to sham stimulation. It was also demonstrated that their auditory hallucinations became less salient in these patients. Importantly, 52% of patients maintained improvement for up to 15 weeks (Hoffman, Hawkins et al. 2003). Fitzgerald et al (2005) also examined the effect of 10 sessions of 1 Hz rTMS over the electrode TP3 of the international 10-20 system for EEG placement on auditory hallucinations in patients with schizophrenia. In this study, however, no improvements were observed in auditory hallucination rating and no improvement or deterioration was reported in cognitive testing (Fitzgerald, Benitez et al. 2005). There are only a couple of studies that have used rTMS to specifically examine its effect on cognition in patients with schizophrenia. First, in a pilot study
based on 4 patients with schizophrenia, Sachdev et al (2005) applied 15 Hz rTMS over the left DLPFC for 4 weeks and reported no significant improvement in general cognitive state, executive function (working memory, verbal fluency, cognitive flexibility) or psychomotor speed (Sachdev, Loo et al. 2005). Second, Huber et al (2003) applied 20 Hz rTMS over the left DLPFC for 2 weeks in 12 patients with schizophrenia that resulted in an improvement in psychomotor speed in women but not men (Huber, Schneider et al. 2003). Together these studies show some potential with high frequency rTMS applied to the DLPFC in improving auditory hallucinations and cognitive deficits in patients with schizophrenia; however, there is a need for more studies that examine different rTMS parameters, session duration, the inclusion of a placebo-controlled sham group and healthy subject group with increased sample size.

1.7.3.1 Targeting the DLPFC

As previously described the DLPFC has been implicated in the pathophysiology of schizophrenia in addition to its critical role in working memory. As such, the DLPFC is a key brain area to be targeted in the treatment of cognitive deficits with rTMS. Traditionally, the DLPFC has been localized with the 5-cm rule which involves first stimulating the hot spot for a hand muscle (i.e., abductor pollicis brevis) through single pulse TMS and then measuring 5 cm anterior from this position along a parasagittal line (George, Wassermann et al. 1995; Pascual-Leone, Rubio et al. 1996). Another method for determining this position is using the 10-20 EEG system (Jasper 1958) which places the coil over electrodes F3 and F4 for targeting the left and right DLPFC, respectively (Gerloff, Corwell et al. 1997; Rossi, Cappa et al. 2001). These methods however have been scrutinized for their poor targeting accuracies particularly in regards to inter-subject and inter-rater variability. More recently, researchers and clinicians have turned to MRI neuronavigational techniques to localize the DLPFC thereby decreasing inter-subject variability with the other two methods. However, the DLPFC is a functional brain region and therefore
cannot be identified through anatomical inspection of a T1-weighted MRI image. In this regard, our lab has developed a novel neuronavigational method which first estimates the DLPFC on the cortex based on fMRI coordinates during working memory and then estimates this position on the scalp. This method is advantageous over previous methods because it minimizes inter-subject and inter-rater sources of variability. Furthermore, our method calculates the angle of the TMS coil into the scalp position of the DLPFC. Finally, the advantage of our method is that it can be used to calculate the scalp position for any functional brain region to be targeted with rTMS. The findings of this study represent an important advancement in improving the localization of the DLPFC to optimize rTMS as a potential treatment for cognitive deficits in schizophrenia. This study has been published (Rusjan et al 2010) and has been included as an appendix to this thesis (Appendix A).

1.7.4 Repetitive TMS and Cortical Oscillations

The effect of rTMS on cortical oscillations has yielded some interesting findings. Moreover, these studies demonstrated that high frequency over the left DLPFC modulates cortical oscillations across all frequency ranges and also produce changes in other distal brain regions. For example, 10 Hz rTMS applied to the left DLPFC in healthy subjects was shown to increase delta power in the frontal, central, and parietal brain regions during an EEG session with eyes closed tested 10 minutes post rTMS (Griskova, Ruksenas et al. 2007). In this study, however, subjects were not blind to their rTMS group assignment and although a sham placebo condition was employed, stimulation was applied in a different brain region and at a lower intensity. In this regard, Okamura et al (2001) conducted a study in 32 subjects that were randomized to receive either active or sham 10 Hz rTMS applied over the left prefrontal cortex. Importantly subjects were blind to their rTMS condition and sham stimulation was applied at the same parameters as the active stimulation with the coil angled perpendicular to the left prefrontal cortex (Okamura,
In this study, 10 Hz rTMS applied to the left prefrontal cortex in healthy subjects resulted in a significant increase in peak frequency of EEG across the scalp with no change in absolute power 2 minutes following stimulation. This effect was most robust in the frontal brain region, however, increases were also observed in the temporal region distal from the stimulation site. By contrast, 5 minutes post rTMS stimulation significantly increased absolute power across delta, theta, alpha, beta, and gamma frequency bands with no change in peak frequency (Okamura, Jing et al. 2001). In a similar study using the same rTMS parameters, Jing and Takigawa (2000) observed directed coherence between the frontal and parietal brain regions in both hemispheres immediately following stimulation. Moreover, this effect was found to be most significant in the alpha band with the directed coherence from the frontal to the parietal brain region increasing by 32% even cross hemispheres. This finding demonstrates that distal brain regions can be modulated by rTMS even if they are not directly connected (Jing and Takigawa 2000). It is possible that nonspecific thalamic system and the corpus callosum may be important in mediating the transhemispheric signal transmission (Hamada and Wada 1998), while the brainstem may play a role in the spread of the signals (McIntyre and Goddard 1973; Kievit and Kuypers 1975). It has also been suggested that the effect of rTMS on oscillatory activity may result from a direct cortical effect (Pascual-Leone, Houser et al. 1993). Specifically, Pascual-Leone and colleagues propose that the excitatory intracortical axons are collateral to pyramidal cells and inhibitory interneurons. Furthermore, inhibitory interneurons form feedback loops and project to the pyramidal cells. The balance between excitatory and inhibitory activity is critical in maintaining homeostasis under normal circumstances, however, rTMS upsets this balance with the accumulation of EPSPs in the absence of IPSPs due to the difference in the number of synapses and conduction along myelinated monosynaptic excitatory collaterals (Pascual-Leone, Houser et al. 1993). This proposed mechanism however does not account for
changes found in the Jing and Takigawa study that demonstrated immediate changes in peak frequency that suggests another mechanism may be involved (Jing and Takigawa 2000).

1.8 Outline of Experiments

Chapter 2 will provide a background and rationale for the three studies conducted as part of the PhD program. In this chapter the objectives and hypothesis for these experiments will be outlined. Chapters 3, 4 and 5 represent studies one, two, and three, respectively. These studies represent publications from the work done during the PhD program. Studies one and two have been published. Study three has been submitted for publication. Because these studies have been submitted as standalone articles, material contained in each of these articles may overlap with material presented in other articles in addition to material that was discussed in the Introduction.
2 Overview of Experiments, Hypotheses and Objectives

2.1 Study 1: Evidence for Excessive Frontal Evoked Gamma Oscillatory Activity in Schizophrenia during Working Memory

2.1.1 Background

Oscillatory activity is associated with working memory. Specifically, increases in gamma (30-50 Hz) oscillatory activity has been demonstrated in response to increases in working memory load in epileptic patients (Howard, Rizzuto et al. 2003) and in healthy subjects (Basar-Eroglu, Brand et al. 2007). Furthermore, Basar-Eroglu et al (2007) reported that patients with schizophrenia generate increased gamma oscillatory activity when tested in the N-back at 3 levels (0-2-back) of working memory load. In addition, a relationship between oscillatory activity generated during working memory and clinical symptoms has yet to be demonstrated. The aim of the first study of the thesis, therefore, was twofold: 1) to quantify alterations in frontal evoked oscillatory activity across the 5 frequency bands (i.e., delta, theta, alpha, beta and gamma) in patients with schizophrenia compared to healthy subjects while performing the N-back task administered at 3 levels (1-3-back); 2) to examine the relationship between gamma oscillatory activity, working memory performance, and the severity of clinical symptoms in patients with schizophrenia. The study included 24 medicated patients with schizophrenia or schizoaffective disorder confirmed by the DSM-IV, and 24 healthy subjects. All subjects were right handed (Oldfield 1971). Patients were recruited through the Schizophrenia and Continuing Care Program at the Centre for Addiction and Mental Health (CAMH), while healthy subjects were recruited through poster advertisement placed at CAMH and the University of Toronto.
2.1.2 Primary Objectives

First, we will compare frontal evoked oscillatory activity in patients with schizophrenia compared to healthy subjects while performing the N-back task. Second, we will examine the relationship between gamma oscillatory activity, performance, and severity of clinical symptoms in patients with schizophrenia.

2.1.3 Primary Hypotheses

We first hypothesized that patients with schizophrenia will exhibit altered frontal evoked gamma oscillatory activity while performing the N-back task across working memory load. Second, we hypothesized that altered gamma oscillatory activity will be related to impaired working memory performance and severity of clinical symptoms.

2.1.4 Results

We found that patients with schizophrenia performed significantly worse in the N-back task compared to healthy subjects that was accompanied by alterations in oscillatory activity. Specifically, we confirmed the findings of Basar-Eroglu (2007) and demonstrated that patients with schizophrenia, compared to healthy subjects, generate excessive frontal evoked gamma oscillatory (F=4.561, p=0.039) regardless of working memory load. Furthermore, we found that this effect was still present at equivalent performance levels (t=-2.898, p=0.006) (Barr, Farzan et al. 2010). Finally, we demonstrate an inverse relationship between working memory performance and negative but not positive symptoms (r=-0.416, p=0.043). These results suggest that gamma oscillatory activity is excessive in patients with schizophrenia and is not modulated by working memory load in contrast to healthy subjects.

The study raised two important questions. First, can high frequency rTMS modulate frontal evoked gamma oscillations generated during the N-back in healthy subjects and in patients with
schizophrenia? Second, if rTMS indeed modulate frontal evoked gamma oscillatory activity, would these neurophysiological changes be reflected in performance improvement? Study two of this thesis was intended to measure the effect of high frequency rTMS applied to the DLPFC on frontal evoked gamma oscillatory activity generated during the N-back in healthy subjects. Study three of this thesis then looked to examine if excessive frontal evoked gamma oscillations in patients with schizophrenia observed in study one could be inhibited by rTMS applied to the DLPFC.

### 2.2 Study 2: Potentiation of Gamma Oscillatory Activity through Repetitive Transcranial Magnetic Stimulation (rTMS) of the Dorsolateral Prefrontal Cortex

#### 2.2.1 Background

From study one it was observed that patients with schizophrenia generate excessive frontal evoked gamma oscillations while performing the N-back task. Furthermore, this effect was accompanied by deficits in working performance which was then found to be related to the severity of negative symptoms. It is therefore important to explore whether frontal gamma oscillations can be influenced with high frequency rTMS applied over the DLPFC.

Repetitive (TMS) has been previously shown to improve cognition in other patient populations. For example, improvements in language function with rTMS has been demonstrated in healthy subjects (Sparing, Mottaghy et al. 2001) and in patients with major depressive disorder (Little, Kimbrell et al. 2000; Martis, Alam et al. 2003; O'Connor, Jerskey et al. 2005). In addition, high frequency rTMS has been shown to enhance GABAergic inhibitory neurotransmission in healthy subjects (Daskalakis, Moller et al. 2006). Given the evidence for the role of GABAergic inhibitory neurotransmission in generation (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007) and modulation (Whittington, Traub et al. 1995; Brown, Davies...
et al. 2007; Leung and Shen 2007) of gamma oscillations, rTMS may exert its influence on gamma oscillatory activity through the modulation of GABAergic activity.

This study included 22 right-handed (confirmed with Oldfield Handedness Inventory (Oldfield 1971) healthy subjects comprised of equal males and females. Subjects were recruited through poster advertisement placed at CAMH and the University of Toronto. This was a blind, placebo-controlled pre-post study design. Subjects were randomly assigned to receive either active or sham (placebo) rTMS stimulation and were blind to their group assignment. All subjects completed the N-back task one week before rTMS was applied to the DLPFC followed by a second testing of the N-back task. The effect of rTMS on frontal evoked oscillatory activity during the N-back task was measured with EEG.

2.2.2 Primary Objective

We will evaluate the effect of 20 Hz rTMS applied bilaterally to the DLPFC on frontal evoked gamma oscillatory activity measured during the N-back in healthy subjects.

2.2.3 Primary Hypothesis

We hypothesize that active rTMS would enhance frontal evoked gamma oscillatory activity in response to increased working memory load and this effect would be absent in activities in the other frequency bands (i.e., delta, theta, alpha, and beta).

2.2.4 Results

Our results demonstrate that rTMS significantly increases gamma oscillations generated during the N-back task in healthy subjects (Barr, Farzan et al. 2009). That is, active rTMS results in greater gamma oscillatory activity compared to baseline (prior to rTMS) (p<0.0001) and to sham stimulation (p=0.0028). Moreover, these effects were most pronounced at higher working
memory loads (2-back: \( p=0.0032 \); 3-back: \( p=0.0288 \)). Importantly, the effect of active rTMS on gamma oscillatory was found to be selective to activity in the gamma frequency range and were isolated to the frontal brain region when compared to the posterior brain region (\( t=4.101, p<0.005 \)). These results therefore demonstrate that rTMS applied to the DLPFC can modulate gamma oscillatory activity a finding that could be translated into patients with schizophrenia.

2.3 Study 3: The Effect of Transcranial Magnetic Stimulation on Gamma Oscillatory Activity in Schizophrenia

2.3.1 Background

Study one of this thesis demonstrated that patients with schizophrenia generate excessive gamma oscillatory activity while performing the N-back task compared to healthy subjects. Study two demonstrated that high frequency rTMS applied bilaterally to the DLPFC potentiates frontal evoked gamma oscillatory activity in healthy subjects that most pronounced in the N-back conditions with the greatest cognitive demands. The findings of these two studies suggest that rTMS over the DLPFC may potentially modulate excessive gamma oscillatory activity observed in patients with schizophrenia while performing the N-back task.

This study tested 24 medicated patients with a diagnosis of schizophrenia or schizoaffective disorder confirmed by the Structural Clinical Interview for DSM-IV (Spitzer 1994). Patients were recruited through the Schizophrenia and Continuing Care Program at CAMH. The healthy control group consisted of 22 subjects who were recruited through poster advertisement placed at CAMH and the University of Toronto. All subjects were right-handed (Oldfield 1971). This study was a randomized, double-blind placebo-controlled design. All subjects were allocated to
receive active or sham stimulation and the subjects and raters were blind to their rTMS condition.

2.3.2 Primary Objective

The aim of this study was to examine the effect of high frequency rTMS applied bilaterally to the DLPFC on frontal gamma oscillatory activity generated during the N-back in patients with schizophrenia compared to healthy subjects.

2.3.3 Primary Hypothesis

It was hypothesized that rTMS would inhibit excessive gamma oscillatory activity in patients with schizophrenia compared to sham stimulation and in contrast to healthy subjects owing to the fact that rTMS has been previously shown to result in the potentiation of GABA inhibitory neurotransmission in healthy subjects (Daskalakis, Moller et al. 2006; Jung, Shin et al. 2008).
Study One: Evidence for Excessive Frontal Evoked Gamma Oscillatory Activity in Schizophrenia during Working Memory

3.1 Abstract

Gamma (γ) oscillations (30-50 Hz) elicited during working memory (WM) are altered in schizophrenia (SCZ). However, the nature of the relationship between evoked frontal oscillatory activity, WM performance and symptom severity has yet to be ascertained. This study had two objectives. First, to extend previous studies by examining delta, theta, alpha, beta, and gamma (δ, θ, α, β, and γ) oscillatory activities during the N-back task in SCZ patients compared to healthy subjects; second, to evaluate the relationship between oscillatory activities elicited during the N-back, performance, and clinical symptoms in SCZ patients. Patients with SCZ elicited excessive frontal γ oscillatory activity that was most pronounced in the 3-back condition compared to healthy subjects. Reduced frontal β activity at all WM loads was also observed in patients with SCZ compared to healthy subjects. Task performance was inversely correlated with negative symptoms but not with positive symptoms. Our findings suggest that evoked frontal oscillatory activities during WM are selectively altered in the γ and β frequency bands that may contribute to WM impairment in SCZ patients. These findings may provide important insights into the pathophysiology underlying WM deficits, its relationship to negative symptoms and may represent a potential neurobiological marker for cognitive enhancing strategies in SCZ.

3.2 Introduction

Gamma (γ) oscillations (30-50 Hz) have been shown to be a key neurophysiological mechanism underlying working memory (WM), a cognitive process involving the maintenance and
manipulation of information on-line (Baddeley 1986). Increased γ oscillatory activity with increased WM load has been demonstrated in epileptic patients (Howard, Rizzuto et al. 2003) and healthy subjects (Basar-Eroglu, Brand et al. 2007) with electroencephalography (EEG) especially in the dorsolateral prefrontal cortex (DLPFC). Although γ modulation in the DLPFC is most consistently associated with WM, delta (δ; 1-3.5 Hz), theta (θ; 4-8 Hz), alpha (α; 9-12 Hz), and beta (β; 14-28 Hz) activities also vary with WM load (Barr, Farzan et al. 2009).

Altered oscillatory activity during WM has been demonstrated in SCZ. Abnormal δ (Ince, Pellizzer et al. 2009), θ (Schmiedt, Brand et al. 2005; Haenschel, Bittner et al. 2009), α (Bachman, Kim et al. 2008; Stephane, Ince et al. 2008; Ince, Pellizzer et al. 2009) and β (Stephane, Ince et al. 2008) activation during WM has been shown in SCZ patients. However, inconsistent reports reveal both similar (Haenschel, Bittner et al. 2009) and abnormally increased (Basar-Eroglu, Brand et al. 2007) γ oscillatory activity during WM in SCZ compared to healthy subjects. Furthermore, Basar-Eroglu et al (2007) demonstrated that SCZ patients failed to modulate γ oscillatory activity with WM load as found in healthy subjects (Basar-Eroglu, Brand et al. 2007). The lack of γ modulation with WM load in SCZ patients may be attributed to alterations in γ–aminobutyric acid (GABA) interneurons in the DLPFC (Akbarian, Kim et al. 1995; Benes and Berretta 2001; Lewis, Hashimoto et al. 2005; Hashimoto, Bazmi et al. 2008) critical to γ oscillatory activity. Considering the importance of GABA in the generation (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007) and synchronization (Whittington, Traub et al. 1995; Brown, Davies et al. 2007) of γ oscillations, GABAergic impairments in patients with SCZ may underlie altered modulation of γ oscillatory during WM.
The relationship between γ oscillatory activity, WM performance, and clinical symptoms has yet to be determined. In a behavioural study, Park et al (1999) reported on a relationship between WM performance and negative symptoms in SCZ patients, such that increased negative symptoms related to poor WM (Park, Puschel et al. 1999). Additionally, a fMRI study in SCZ patients demonstrated a correlation between strength of prefrontal-parietal connectivity and WM performance, while positive symptoms related to decreased connectivity (Henseler, Falkai et al. 2009). Relationships have also been uncovered between γ oscillatory activity with both positive and negative symptoms although through an auditory odd-ball task and not WM (Lee, Williams et al. 2003). In this study, patients were divided by symptom predominance and found that positive symptoms correlated with increased γ, while negative symptoms were related to a reduction of γ (Lee, Williams et al. 2003). As such, γ oscillations during WM may be related to both performance and symptom severity in SCZ patients. In the current study, therefore, we aimed to extend previous findings by examining evoked oscillatory activity during WM across δ, θ, α, β, and γ frequency components measured from the frontal region in SCZ patients compared to healthy subjects. We also sought to examine if neuronal oscillations during WM were related to task performance and symptom severity.

3.3 Materials and Methods

3.3.1 Subjects

Twenty-four (males=14; females=10) patients with a diagnosis of SCZ or schizoaffective disorder (SCZ=19; schizoaffective disorder=5), confirmed by the Structured Clinical Interview for DSM-IV (Spitzer 1994), and 24 (males=13; females=11) healthy subjects participated in this study. All subjects were right handed (Oldfield 1971). SCZ patients were treated with antipsychotic medication (15.0 ± 14.1 mg olanzapine, 5 patients; 318.8 ± 196.3 mg clozapine, 8
patients; 3.7 ± 1.5 mg risperidone, 3 patients; 650.0 ± 378.6 mg of quetiapine, 4 patients; 0.7 ±
0.8 mg of fluphenazine, 2 patients; 5 mg haloperidol, 1 patient; 15 mg aripiprazole, 1 patient;
dose/day). Severity of psychopathology was evaluated using the positive and negative symptom
scale (PANSS; (Kay, Fiszbein et al. 1987)), scale for the assessment of negative symptoms
(SANS; ((Andreasen 1989)) and the Calgary Depression Scale (CDS; ((Addington, Addington et
al. 1993); Table 1). Subject groups were similar in age ($t_{(45)}=0.320$, $p=0.750$), but differed in
education (independent t-tests: $t_{(45)}=14.771$, $p<0.001$; Table 1). Exclusion criteria for all
subjects included a history of substance abuse or dependence in the last 6 months determined
through the DSM-IV or pregnancy. Healthy subjects were excluded if a concomitant major and
unstable medical, neurologic illness and/or the presence of psychopathology determined by the
personality assessment screener (PAS; Psychological Assessment Resources, Inc). Subjects
provided their written informed consent and the protocol was approved by the Centre for
Addiction and Mental Health in accordance with the declaration of Helsinki.

3.3.2 N-Back Task

Subjects performed the N-back task while EEG activity was recorded (STIM2, Neuroscan,
U.S.A.). Stimuli were presented on a computer monitor one at a time and participants were
required to push one button (target) if the present stimulus was identical to the stimulus
presented “N” trials back; otherwise, subjects pushed a different button (non-target). Thus, the
effect of increasing cognitive demand on γ oscillatory activity was tested by varying the “N” in
the 1-, 2- and 3-back conditions. Stimuli consisted of black capital letters presented for 250
msec followed by a delay period of 3000 msec during which the subject was required to respond
(Figure 1). In the 1-, and 2-back, stimuli were presented continuously for 15 minutes and for 30
minutes in the 3-back. The 3-back was administered for double the length of time to ensure a
satisfactory number of correct responses were contained for the data analysis (Table 2). The
number of target letters in each condition was: 46 of 198 (23.2%) 1-back; 31 of 197 trials (15.7%) 2-back, 59 of 400 trials (14.6%) 3-back condition. The N-back took 1 hour to complete with the order of conditions randomized within subjects and counterbalanced across subjects to prevent order effects.

3.3.3 EEG Measurement of Evoked γ Oscillatory Activity

Evoked oscillatory responses are phase-locked to the stimulus onset with a fixed latency within the first 100 msec following stimulus onset and are measured by stimulus-triggered averaging of responses (Tallon-Baudry and Bertrand 1999). While, induced oscillatory activity is not phase-locked to stimulus onset and jitters in latency varying from trial to trial; thus, responses are cancelled out when averaged (Tallon-Baudry and Bertrand 1999). Although both evoked and induced γ activities have been associated with WM (Howard, Rizzuto et al. 2003; Basar-Eroglu, Brand et al. 2007; Barr 2008), induced γ activity has been suggested to reflect microsaccadic eye movement rather than neuronal processing during cognitive paradigms (Yuval-Greenberg, Tomer et al. 2008). Additionally, γ activity during cognitive tasks has been shown to reflect cranial musculature artifact (Shackman 2010), however, since this activity is characterized by irregular spikes and waves present in all spectral frequencies such artifact should be significantly reduced when multiple trials are averaged with evoked analytical methods (Barr and Daskalakis 2010). As such, we measured mean evoked γ power from frontal electrodes while subjects completed the N-back task.

3.3.4 EEG Recording

EEG data were acquired using a 64-electrode cap and Synamps2 DC-coupled EEG system (Compumedics, U.S.A.). Four electrodes placed on the outer side of each eye, above, and below the left eye to monitor eye movement artifact. Data was recorded at a rate of 1000 Hz DC and
with a 0.3 to 200 Hz band pass hardware filter. Electrode impedances were lowered to < 5 kΩ. All channels were referenced to an electrode placed posterior to the Cz electrode.

### 3.3.5 Offline EEG processing

Data was filtered off-line using a 1 to 100 Hz band pass zero phase shift filter (slope, 24 dB/oct). Epochs were defined as –1000 to +3095 msec relative to the cue onset and were baseline corrected with respect to the prestimulus interval (-1000 to cue onset). Trials were manually inspected and any error trials or epochs containing artifact (movement or electrooculogram exceeding +/- 50 µV) were excluded from further analysis. On average, we excluded 26% of all correct trials (TC+NTC) from the healthy subject group and 27% from the SCZ patient group. Finally, oscillatory power was extracted by decomposing the signals by means of a hamming-based zero-phase shift finite impulse response filter.

### 3.3.6 Data Analysis

The total number of correct trials (target correct (TC) and non-target correct (NTC)) including those trials rejected due to artifact were included in the data analysis for WM performance and reaction time. Two separate repeated measures ANOVA for WM and reaction were performed with Group (SCZ versus healthy subjects) as a between-subject factor with WM load (1 versus 2 versus 3) as a within-subject factor, significance level set at p<0.05. The assumption of sphericity was met with each repeated measures ANOVA. Subsequent pairwise comparisons were performed with the level of significance Bonferroni-adjusted (SPSS 15.0, SPSS Inc. Chicago, Illinois, USA).

Artifact-free EEG data were imported into MATLAB (The MathWorks, Inc. Natick, MA, USA) using the EEGLAB toolbox (Delorme and Makeig, 2004) for subsequent analysis. Evoked γ power (30-50 Hz) was averaged over the delay period for TC and NTC responses for each WM
load. Mean evoked power during these responses (TC + NTC) in the frontal electrodes (AF3/4, F5/6, F3/4, F1/2, and FZ) were measured and then averaged. Since spectral analysis of EEG activity is often not normally distributed (Bender, Schultz et al. 1992), the data was log transformed prior to analysis. For each frequency (δ, 1-3.5 Hz; θ, 4-8 Hz; α, 9-12 Hz; β, 14-28 Hz; and γ, 30-50 Hz), a repeated measures analysis of variance (ANOVA) was performed with Group (SCZ versus healthy subjects) as a between-subject factor with WM load (1 versus 2 versus 3) as a within-subject factor, significance level set at p<0.05. The assumption of sphericity was met with each repeated measures ANOVA. Subsequent pairwise comparisons were performed with the level of significance Bonferroni-adjusted. Spearman ranked correlations were used to evaluate the relationship between γ oscillatory activity, performance and clinical symptoms in SCZ patients (SPSS 15.0, SPSS Inc. Chicago, Illinois, USA).

3.4 Results

3.4.1 Working Memory Performance

N-back performance decreased with increased WM load ($F_{(2,104)}=69.678; p<0.0001$; Table 3). Subjects performed significantly worse in 3- compared to 2- and in 2- compared to 1-back condition ($p<0.0001$ in both cases). The Group difference in N-back performance was also significant ($F_{(1,44)}=9.533; p=0.004$) with SCZ patients performing significantly worse compared to healthy subjects. Mean reaction time increased with increased WM load ($F_{(2,88)}=17.263; p<0.0001$; Table 3) that significantly increased from the 1- to 2-back ($p<0.0001$) and trended towards significance from the 2- to the 3-back condition ($p=0.092$).

3.4.2 Evoked γ Power

The effect of WM load on mean γ power was significant ($F_{(2,84)}=3.820; p=0.026$) and was greatest in the 2-back (Figure 2A). The Group x WM load interaction was also significant ($F_{(2,84)}$...
= 10.537; \( p < 0.001 \) ), independent t-tests revealed that SCZ patients elicited higher \( \gamma \) power at each WM load that reached significance in the 3-back condition (\( p < 0.001 \); shown as Topographical plots Figure 2B). In healthy subjects, \( \gamma \) power increased from 1- to the 2-back and decreased in the 3-back (\( p = 0.001 \) in each case). Conversely, no differences were observed between N-back conditions in SCZ patients (\( p > 0.05 \) in each case), reflecting a lack of \( \gamma \) modulation with WM load. The Group effect was significant (\( F_{(1,42)} = 4.561; p = 0.039 \)) with SCZ patients generating excessive \( \gamma \) activity overall. At equivalent performance levels, \( \gamma \) oscillatory activity was compared between the two groups. An independent t-test first determined that SCZ 1-back performance was equivalent to 3-back performance healthy subjects (\( t_{(44)} = -0.941, p = 0.352 \)). Next, \( \gamma \) activities were compared in these conditions and found that SCZ patients still elicited significantly greater \( \gamma \) oscillatory activity compared to healthy subjects at equivalent performance levels (\( t_{(45)} = -2.898, p = 0.006 \)). SCZ patients, therefore, elicited excessive \( \gamma \) oscillatory activity independent of WM load compared to healthy subjects.

### 3.4.3 Spectral Analysis of Other Frequency Bands

To test whether alterations in SCZ patients was selective to the \( \gamma \), we examined oscillatory power in the following four bands: \( \delta \), \( \theta \), \( \alpha \), and \( \beta \) compared to the healthy subjects. No group differences were found in \( \delta \), \( \theta \), and \( \alpha \); however, SCZ patients elicited significantly reduced \( \beta \) activity compared to healthy subjects (\( F_{(1,40)} = 15.859, p < 0.0001 \)) that did not vary with WM load (Figure 3).

### 3.4.4 Relationship Between Evoked Power, N-back Performance, and Symptom Severity

Spearman ranked correlations were performed to examine the relationship between \( \gamma \) power in the 3-back with performance and clinical symptoms owing to the fact that group differences in \( \gamma \)
power in this condition were most pronounced. An inverse relationship was found between negative symptoms measured by the SANS and working memory performance \(r=-0.538\), \(p=0.007\). That is, the greater the severity of negative symptoms the worse SCZ patients performed in the 3-back condition.

### 3.4.5 Effect of Antipsychotic Medication

A Pearson correlation coefficient was performed to determine if excessive \(\gamma\) and reduced \(\beta\) oscillatory activity in the 3-back was related to antipsychotic medication using chloropromazine equivalents (CPZ; (Woods 2003)). No relationships were found between \(\gamma\) or \(\beta\) oscillatory activities and CPZ equivalents in the 3-back. Further, no relationship was observed between 3-back performance and CPZ equivalents examined through Pearson correlation coefficient.

### 3.5 Discussion

SCZ patients generated excessive evoked frontal \(\gamma\) oscillatory activity that was most pronounced in the 3-back compared to healthy subjects. Task performance was inversely correlated with negative symptoms but not with positive symptoms. Finally, there was a reduction in \(\beta\) oscillatory activity in SCZ patients, but this was not related to symptom severity, task performance or WM load.

Consistent with Basar-Eroglu et al (2007), we observed an increase in frontal \(\gamma\) oscillatory activity at each WM load, particularly in the 3-back condition in SCZ patients compared to healthy subjects. Although in the Basar-Eroglu study, the N-back task was administered up until the 2-back condition, we observed the most pronounced effects in the 3-back condition. Such an effect is, in part, driven by the decrease in frontal \(\gamma\) oscillatory activity in the 3-back relative to the 2-back condition in healthy subjects. This inverted u-shaped curve in response to increased
WM load is consistent with fMRI studies (Callicott, Mattay et al. 1999) reflective of poorer WM performance possibly due to diminished attentional resources (Cowan 2001; Kane, Bleckley et al. 2001; Wheeler and Treisman 2002). Nevertheless, we observed excessive frontal $\gamma$ oscillatory activity in SCZ patients regardless of WM load suggesting a lack of $\gamma$ modulation in response to increased cognitive demand. This finding is further substantiated by the fact that SCZ patients’ frontal $\gamma$ oscillatory activity is significantly greater compared with healthy subjects at equivalent performance levels and, therefore, was not due to poorer performance. Possibly this phenomenon is related to altered GABAergic inhibitory neurotransmission in the DLPFC.

There are, in fact, several lines of evidence to support this contention. First, post-mortem studies have demonstrated reductions in the 67 kilodalton isoform of glutamic acid decarboxylase ($\text{GAD}_{67}$) (Akbarian, Kim et al. 1995; Benes and Berretta 2001; Volk, Pierri et al. 2002; Lewis, Hashimoto et al. 2005) and calcium binding protein parvalbumin in the DLPFC (Collin, Chat et al. 2005). Second, reduction of parvalbumin leading to increased facilitation of GABA release during repetitive synaptic firing has been directly linked $\gamma$ modulation (Vreugdenhil, Jefferys et al. 2003; Sohal, Zhang et al. 2009). Third, a recent study measured neurophysiological indices of $\text{GABA}_B$ receptor inhibitory neurotransmission from the DLPFC in SCZ compared to patients with bipolar disorder and healthy subjects through combined TMS-EEG (Farzan, Barr et al. 2010). Waveforms were decomposed into frequency components and demonstrated that inhibition of $\gamma$ oscillations were significantly reduced in DLPFC in SCZ compared to the other two groups (Farzan, Barr et al. 2010). It is possible that the lack of inhibition of $\gamma$ oscillatory activity in SCZ patients results in excessive $\gamma$ oscillations during WM that may contribute to performance deficits in this disorder.
Consistent with previous studies, we observed reduced β oscillatory activity in SCZ patients. For example, SCZ studies have demonstrated β band reductions in phase synchrony in visual perception of Mooney faces (Uhlhaas, Linden et al. 2006), inter-trial coherence preceding speech generation (Krishnan, Vohs et al. 2005), and in steady-state visual evoked potentials (Krishnan, Vohs et al. 2005). Such altered β oscillations may be related to GABA_B activity, as GABA_B agonist L-baclofen administered at low doses (5mg/kg) increases β and decreases γ power, while at higher doses (50 mg/kg) a reduction in β and an increase in γ power was observed in mice (Marrosu, Santoni et al. 2006). The differential effect of L-baclofen on γ and β oscillatory activity their study is congruent with the finding that such activities are synchronized by different cellular mechanisms (Traub, Whittington et al. 1999). Furthermore, studies have reported on the phenomenon of a γ to β frequency shift in animal models (Whittington, Stanford et al. 1997) and in SCZ (Hong, Summerfelt et al. 2004). Specifically, Hong et al (2004) examined the suppression of auditory evoked potentials using the P50 paradigm in SCZ patients compared to healthy subjects. This paradigm involves two auditory clicks (S1, S2), and with normal sensory gating the magnitude of the P50 response to S2 is attenuated compared to S1 response. In healthy subjects, a shift from γ to β activity in response to S1 was found to underlie the suppression of S2 (Hong, Summerfelt et al. 2004). That is, the level of β activity following S1, the greater the P50 response. By contrast, the shift from γ to β activity following S1 was reduced in SCZ patients leading to decreased P50 responses (Hong, Summerfelt et al. 2004). Together these studies support the coupling of γ and β activities that may explain the associated elevation in γ and reduction in β power elicited during WM in this study.

In SCZ patients, N-back performance was inversely related to the severity of negative symptoms. This finding is consistent with several cross-sectional studies that have demonstrated a correlation between cognitive performance and negative symptoms (Harvey, Howanitz et al.
Moreover, it has been argued that SCZ patients with the greatest cognitive impairment present the most prominent negative symptoms (Basso et al. 1998; Villalta-Gil et al. 2006). It has further been suggested that negative symptoms mediate the relationship between cognition and functional outcome (Ventura et al. 2009).

To our knowledge, this is the first study that has shown excessive γ with a concomitant reduction in β oscillatory activity during WM in SCZ. Furthermore, excessive γ oscillations were related to depressive symptoms, while N-back performance was related to negative symptoms. However, this study is limited in several ways. First, our finding of excessive γ oscillations in SCZ may be attributed to the effects of antipsychotic medication. In this regard, Hong et al. (2004) reported enhanced 40 Hz oscillations in SCZ patients on second generation compared to those taking conventional antipsychotics (Hong et al. 2004). In their study, oscillations were parsed into 20, 30 and 40 Hz; however, 30-50 Hz range is most conventionally examined during cognitive tasks (Howard, Rizzuto et al. 2003). Similarly, a differential effect of antipsychotic medication on cognitive performance has also been reported with SCZ patients on second generation antipsychotics performing better than those patients on conventional antipsychotics on a variety of cognitive tests, including WM (Meltzer and McGurk 1999; Bilder, Goldman et al. 2002; Sharma, Hughes et al. 2003). In our sample, 5 subjects were on conventional antipsychotics, but no differences were found in γ or WM performance compared to patients on second generation antipsychotics. Furthermore, there was no relationship between γ, β or performance in the 3-back condition with CPZ equivalents. Second, the N-back task does not allow for examination of oscillatory activity within the different sub-processes in WM (i.e., encoding, maintenance, retrieval). In this regard, Haenschel et al. (2009) reported reductions in evoked θ, α, and β activities during encoding, a peak shift of induced γ activity during
maintenance, and reduced induced θ and γ activities during retrieval (Haenschel, Bittner et al. 2009). However, no differences in evoked or induced γ oscillatory activity in the frontal region were observed in this study.

In summary, alterations in oscillatory activity during WM are selective to the γ and β activation independent of WM load in SCZ. N-back performance was also related to greater negative symptom severity. These findings may provide important insights into the pathophysiology underlying WM deficits, its relationship to clinical symptoms and may represent a potential neurobiological marker for cognitive enhancing strategies in SCZ.
**Table 1.** Demographic data (±) 1 standard deviation in healthy subjects (HS) versus patients with schizophrenia (SCZ) and the assessment of psychotic symptoms in SCZ patients.

<table>
<thead>
<tr>
<th></th>
<th>HS</th>
<th>SCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>37.71 (±) 10.12</td>
<td>37.09 (±) 11.04</td>
</tr>
<tr>
<td><strong>Age Range</strong></td>
<td>24-60</td>
<td>23-57</td>
</tr>
<tr>
<td><strong>Female (n)</strong></td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><strong>Male (n)</strong></td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td><strong>PANSS Scores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>NA</td>
<td>15.79 (±) 6.87</td>
</tr>
<tr>
<td>Negative</td>
<td>NA</td>
<td>15.58 (±) 8.49</td>
</tr>
<tr>
<td>Global</td>
<td>NA</td>
<td>25.83 (±) 9.43</td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>55.50 (±) 20.71</td>
</tr>
<tr>
<td><strong>CDS Score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>2.92 (±) 3.16</td>
</tr>
<tr>
<td><strong>SANS Score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>38.54 (±) 22.40</td>
</tr>
</tbody>
</table>
**Table 2.** Working memory (WM) behavioural data in healthy subjects (HS) versus patients with schizophrenia (SCZ) in the 1-, 2-, and 3-back task conditions (±) 1 standard deviation. Behavioural data was calculated on all trials that were correct including those trials that were rejected due to artifact.

<table>
<thead>
<tr>
<th>WM Behavioural Data</th>
<th>N-Back Condition</th>
<th>HS</th>
<th>SCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM Score (% Correct)</td>
<td>1-Back</td>
<td>90.79 (±) 7.51</td>
<td>79.19 (±) 19.50</td>
</tr>
<tr>
<td></td>
<td>2-Back</td>
<td>80.80 (±) 19.99</td>
<td>70.29 (±) 20.53</td>
</tr>
<tr>
<td></td>
<td>3-Back</td>
<td>73.56 (±) 21.08</td>
<td>55.03 (±) 19.23</td>
</tr>
<tr>
<td>Reaction Time (msec)</td>
<td>1-Back</td>
<td>729.50 (±) 162.74</td>
<td>743.04 (±) 311.66</td>
</tr>
<tr>
<td></td>
<td>2-Back</td>
<td>904.11 (±) 255.50</td>
<td>859.63 (±) 333.73</td>
</tr>
<tr>
<td></td>
<td>3-Back</td>
<td>981.08 (±) 293.34</td>
<td>870.46 (±) 324.32</td>
</tr>
</tbody>
</table>
Figure 1. A) A representation of the 1-, 2- and 3-back conditions that were completed in a randomized order by patients with schizophrenia (SCZ) and healthy subjects. Subjects were required to push one button (target) if the current letter was identical to the letter presented “N” trials back; otherwise the participants pushed a different button (non-target). Correct responses for target (TC) and non-target (NTC) were included in the data analysis. B) The timing of one trial from the presentation of a one letter separated by a (+) sign followed by a subsequent letter for a total time of 3000 msec.
Figure 2. A) Mean log transformed $\gamma$ (30-50 Hz) oscillatory power (TC+NTC) elicited across N-back task conditions in patients with schizophrenia (SCZ) compared to healthy subjects (HS). Error bars represent ($\pm$) 1 standard error (SE). B) Topographical illustration of mean absolute $\gamma$ power elicited during 3-back in patients with SCZ compared to HSs. Greater $\gamma$ power is represented by hot colours.
Figure 3. Mean log transformed frequency power in the δ (1-3.5 Hz), θ (4-7 Hz), α (8-12 Hz), and β (12.5-28 Hz) ranges (TC+NTC) elicited across N-back task conditions in patients with schizophrenia (SCZ) compared to healthy subjects (HS). Error bars represent (±) 1 standard error (SE).
4 Study 2: Potentiation of Gamma Oscillatory Activity through Repetitive Transcranial Magnetic Stimulation (rTMS) of the Dorsolateral Prefrontal Cortex

4.1 Abstract

Neuronal oscillations in the gamma (γ) frequency range (30-50 Hz) have been associated with cognition. Working memory (WM), a cognitive task involving the on-line maintenance and manipulation of information, elicits increases in γ oscillations with greater cognitive demand, particularly in the dorsal lateral prefrontal cortex (DLPFC). The generation and modulation of γ oscillations have been attributed to inhibitory interneuron networks that use γ-aminobutyric acid (GABA) as their principle neurotransmitter. Repetitive transcranial magnetic stimulation (rTMS) represents a non-invasive method to stimulate the cortex that has been shown to modify cognition and GABA inhibitory mechanisms, particularly with higher frequencies (i.e., 10-20 Hz). We measured the effect of high-frequency rTMS over the DLPFC on γ oscillations elicited during the N-back WM task in healthy subjects. Active rTMS significantly increased γ oscillations generated during the N-back conditions with the greatest cognitive demand. Further, no significant changes were found in other frequency ranges, suggesting that rTMS selectively modulates γ oscillations in the frontal brain regions. These findings provide important insights into the neurophysiological mechanisms that underlie higher order cognitive processes, and suggest that rTMS may be used as cognitive enhancing strategy in neuropsychiatric disorders that suffer from cognitive deficits.
4.2 Introduction

The importance of gamma (γ) oscillatory activity in higher cognitive tasks is an area of great interest and has recently been shown as a key neurophysiological mechanism underlying working memory (WM). Commonly defined as the ability to maintain and manipulate information over short periods of time (Baddeley 1986), WM is often argued as the essence of all prefrontal functions due to its importance in everyday complex cognitive tasks, such as language, comprehension, learning, and reasoning (Baddeley 1992; Baddeley 2000). Moreover, the dorsolateral prefrontal cortex (DLPFC) is consistently reported to mediate WM processes revealed through enhanced blood oxygen level dependent (BOLD) activity in functional magnetic resonance imaging (fMRI) studies (Owen 1997; Petrides 2000).

Several cognitive paradigms are used to index WM. For example, the N-back task requires subjects to determine if the current stimulus is the same stimulus that was presented “N” trials back, thus allowing the evaluation of increasing cognitive demand (i.e., WM load) on γ oscillatory activity. Further, a growing body of evidence has demonstrated increased γ oscillatory activity with cognitive demand (Howard, Rizzuto et al. 2003; Basar-Eroglu, Brand et al. 2007; Meltzer, Zaveri et al. 2008). For example, Cho and colleagues (2006) reported that γ oscillatory activity increased with increased cognitive control in the frontal regions that was related to performance (Cho, Konecky et al. 2006) suggesting that γ is modulated by cognitive demand, which may underlie WM performance. It has been proposed that γ-aminobutyric acid (GABA) inhibitory interneurons in the DLPFC contribute to the generation and synchronization of pyramidal neurons necessary for optimal WM performance (Wang and Buzsaki 1996; Traub, Michelson-Law et al. 2004). For instance, Wilson and colleagues (1994) reported fast-spiking GABAergic neuron activity in the DLPFC during the delay period of WM tasks (Wilson,
O'Scalaidhe et al. 1994), while the injection of GABA antagonist bicuculline disrupts WM performance in monkeys (Sawaguchi, Matsumura et al. 1989).

Repetitive transcranial magnetic stimulation (rTMS) delivers repeated magnetic pulses to the cortex to induce plasticity-like changes in cortical function and behaviour. For example, high-frequency rTMS has been shown to improve language functions in healthy individuals (Sparing, Mottaghy et al. 2001), and improve different aspects of memory in major depressive disorder patients (Little, Kimbrell et al. 2000; Martis, Alam et al. 2003; O'Connor, Jerskey et al. 2005). Additionally, rTMS over the motor cortex has been shown to enhance GABA-mediated inhibitory neurotransmission in healthy individuals (Daskalakis, Moller et al. 2006). That is, Daskalakis and colleagues reported a lengthening of the cortical silent period—a measure reflective of GABA$_B$-mediated inhibitory neurotransmission (Ziemann, Lonnercker et al. 1996) with increased stimulation frequency, that was maximal at 20 Hz (Daskalakis, Moller et al. 2006). High-frequency (i.e., 20 Hz) rTMS, thus, represents a possible mechanism through which to potentiate $\gamma$ oscillatory activity that may, in turn, improve WM performance.

This study, therefore, aimed to evaluate the effect of 20 Hz rTMS applied to the right and left DLPFC on $\gamma$ oscillatory activity elicited during the N-back task in healthy subjects. We hypothesized that active rTMS would enhance $\gamma$ oscillatory activity with increased WM load with no effect on oscillatory activity in other frequency ranges (i.e., $\delta$, $\theta$, $\alpha$, and $\beta$).

4.3 Materials and Methods

4.3.1 Subjects

Twenty-two, right-handed healthy volunteers participated in this study (mean age=34.2 years, SD=7.16 years, range=24-49 years; 11 men and 11 woman). Handedness was confirmed using the Oldfield Handedness Inventory (Oldfield 1971). All subjects gave their written informed
consent and the protocol was approved by the Centre for Addiction and Mental Health in accordance with the declaration of Helsinki. Exclusion criteria included a self-reported comorbid medical illness or a history of drug or alcohol abuse. Moreover, psychopathology was ruled out through the personality assessment screener (PAS; Psychological Assessment Resources, Inc).

4.3.2 Procedure

Prior to the experiment, subjects were randomized into two groups allocated to receive either active or sham rTMS. Participants were blind to their group assignment until the completion of the study to avoid subject biases. The experiment took place over two testing days. On the first day, subjects performed the N-back test while their EEG activity was recorded. One week later, rTMS was administered over the DLPFC prior to the final testing in the N-back task. The final N-back task was performed approximately 20 minutes following the rTMS administration to allow for cortical plasticity changes to take place as well as for the placement of the EEG cap. These two N-back testing sessions will be referred to ‘pre’ and ‘post’ measures relative to the rTMS administration here on in.

4.3.3 N-Back Task

Participants performed the N-back task while their EEG activity was recorded (STIM2, Neuroscan, U.S.A.) prior to (pre) and after (post) a single session of rTMS to the DLPFC. During this task, stimuli were presented on a computer monitor one at a time to the participants who were required to push one button (target) if the present stimulus was identical to the stimulus presented “N” trials back, otherwise the participants pushed a different button (non-target). Thus, the effect of increasing the cognitive demand on \( \gamma \) oscillatory activity was tested by varying the “N” of the task in the 0-, 1-, 2- and 3-back conditions (Figure 1). Notably, in the
0-back condition, subjects were required to push the non-target button every time a stimulus was presented, and thus, did not have a memory component. Stimuli consisted of black capital letters presented for 250 msec followed by a delay period of 3000 msec during which the subject was required to respond prior to the presentation of a plus sign for 1305 msec indicating the end of the current trial. In the 0-, 1-, and 2-back condition, stimuli were presented continuously for 15 minutes, while in the 3-back condition; stimuli were presented for 30 minutes. The 3-back condition was administered for double the length of the other conditions (30 minutes rather than 15 minutes) to ensure that the frequency of target letters in this condition was comparable to those presented in the 1-, and 2-back conditions, as these target letters occurred less frequently (i.e., at least 3 letters back). Thus, the proportion of target letters in each of the condition was 23.1%, 15.8%, and 14.8% in the 1-, 2-, and 3-back conditions, respectively. Consequently, this also ensured that a satisfactory number of correct responses were contained in the all of the conditions for the data analysis (Table 1). The total time for participants to complete the N-back task was 1 hour and 15 minutes with the presentation of the conditions randomized and counterbalanced to prevent order effects.

### 4.3.4 Repetitive TMS

Repetitive TMS was administered using a Medtronic MagPro stimulator (Medtronic, Inc., U.S.A.) with a 70 mm diameter figure-of-8 coil to the right and left DLPFC at 20 Hz, 90% resting motor threshold for 25 trains comprising of 30 pulses per train, inter-train interval of 30 seconds for a total of 750 pulses per hemisphere and in accordance with published safety guidelines (Chen, Gerloff et al. 1997). The time of the rTMS delivery was 25 minutes, 12.5 minutes per hemisphere. The resting motor threshold was defined as the lowest intensity that produced a motor evoked potential of at least 50 µV in 50% of the trials delivered. Sham stimulation was delivered with the same rTMS parameters as active stimulation with the coil
held in a single wing-tilt position at 90 degrees to induce similar somatic sensations as in the active stimulation with minimal direct brain effects. The order of stimulation (right then left versus left then right) was also randomized and counterbalanced to prevent order effects.

4.3.5 DLPFC Site Localization

The localization of the DLPFC was determined through neuronavigational techniques using the MINIBIRD system (Ascension Technologies) combined with MRicro/reg software using a T1-weighted MRI scan obtained for each subject with seven fiducial markers in place. Repetitive TMS was targeted at the junction of the middle and anterior one-third of the middle frontal gyrus (Talairach coordinates (x, y, z) = +/- 50, 30, 36) corresponding with posterior regions of Brodmann area 9 (BA9), and overlapping with the superior region of BA46 (Figure 2). The selection of this site was based on a recent meta-analysis of functional imaging studies that examined WM and the activation of the DLPFC (Cannon, Glahn et al. 2005; Mendrek, Kiehl et al. 2005; Tan, Choo et al. 2005).

4.3.6 EEG Measurement of Evoked γ Oscillatory Activity

Evoked oscillatory responses are phase-locked to the stimulus onset with a fixed latency within the first 100 msec following stimulus onset and, therefore, can be measured by stimulus-triggered averaging of responses (Tallon-Baudry, Kreiter et al. 1999). Induced oscillatory activity, on the other hand, is not phase-locked to stimulus onset and appears as a jitter in latency that varies from trial to trial, thus, these responses are cancelled out when trials are averaged (Tallon-Baudry, Kreiter et al. 1999). Both evoked and induced γ activities have been associated with sensory and cognitive processing (Lutzenberger, Pulvermuller et al. 1995; Muller, Bosch et al. 1996; Cho, Konecky et al. 2006). Moreover, induced γ oscillatory activity has been shown to increase with increased cognitive control (Cho, Konecky et al. 2006), while other studies have
shown that evoked γ oscillatory activity increases with cognitive demand in WM paradigms (Howard, Rizzuto et al. 2003; Basar-Eroglu, Brand et al. 2007; Meltzer, Zaveri et al. 2008). As such, we measured mean evoked γ power from frontal electrodes while subjects completed the N-back task before (pre) and after (post) rTMS was administered over the right and left DLPFC.

4.3.7 EEG Recording

EEG data were acquired using a 64-electrode cap and Synamps2 DC-coupled EEG system (Compumedics). Four electrodes placed on the outer side of each eye, above, and below the left eye were used to monitor eye movement artifact. Data was recorded at a rate of 1000 Hz DC and with a 0.3 to 200 Hz band pass hardware filter. Electrode impedances were lowered to < 5 kΩ. All channels were referenced to an electrode placed posterior to the Cz electrode.

4.3.8 Offline EEG processing

To measure the mean evoked γ power, data was filtered off-line using a 1 to 100 Hz band pass zero phase shift filter (slope, 24 dB/oct). Epochs were defined as –1000 to +3095 msec relative to the cue onset and were baseline corrected with respect to the prestimulus interval (-1000 to cue onset). To measure evoked γ power, we selected the entire delay period of 3000 msec relative to stimulus because the N-back task requires continuous maintenance of “N” letters in preparation for the next trial as the task runs sequentially for approximately 15-30 minutes depending on the task condition. All trials were manually inspected and any error trials or epochs containing artifact (movement or electrooculogram exceeding +/- 50 µV) were excluded from further analysis.

4.3.9 Data Analysis

Artifact-free EEG data was imported into MATLAB (The MathWorks, Inc. Natick, MA, USA) using the EEGLAB toolbox (Delorme and Makeig 2004) for subsequent analysis. Evoked γ
power in the 30-50 Hz range was averaged over the delay period (0-3000 msec from cue onset) for the target correct (TC) and non-target correct (NTC) responses for each WM load pre and post rTMS for each participant. Mean evoked γ power was then assessed during these responses (TC + NTC) in the frontal electrodes (AF3, AF4, F5, F3, F1, FZ, F2, F4, and F6), and averaged for each individual. Since spectral analysis of EEG activity is often not normally distributed (Bender, Schultz et al. 1992), the data was log transformed prior to analysis. Full-factorial mixed model repeated measures (MMRM) was performed with rTMS (active versus sham) as a between-subject factor with time (pre versus post) and WM load (0- versus 1- versus 2- versus 3-back) as within-subject factors on the data with a significance level set at p<0.05. Bonferroni-adjusted pairwise comparisons were then performed (SAS System v.9.1.3; SAS Institute, NC, USA).

4.4 Results
4.4.1 Evoked γ Power

As expected, the MMRM analysis revealed an effect of WM load on mean evoked γ power ($F_{(3,60)}=18.59$, $p<0.0001$) with subjects generating the greatest γ power during the 2-back condition compared to the 0- ($p<0.0001$), 1- ($p=0.0416$) and 3-back ($p=0.0004$) conditions, respectively (Figure 3A). Furthermore, the effect of time on γ power was also found significant ($F_{(1,20)}=29.49$, $p<0.0001$) with greater γ power elicited during the N-back following rTMS stimulation (post) compared to baseline (pre). Finally, the effect of rTMS on γ power was also significant ($F_{(1,20)}=9.26$, $p=0.0064$) with subjects in the active group generating higher γ power during the N-back task compared to those who received sham stimulation.

The MMRM analysis on mean γ power also revealed significant interaction effects (Figure 3A). In particular, an interaction was found between time and rTMS ($F_{(1,20)}=12.43$, $p=0.0021$) with
active stimulation resulting in greater $\gamma$ power compared to baseline (pre; $p<0.0001$) and to sham rTMS (post; $p=0.0028$). There was also a significant time x WM load x rTMS interaction: $(F_{(3,60)} = 3.79, p=0.0148)$. Importantly, while there was no difference in $\gamma$ power during the N-back task at baseline (pre) between the active and sham groups, active rTMS resulted in a significant increase in $\gamma$ power in the 2- (p=0.0032) and 3-back (p=0.0288) conditions compared to the sham group. Taken together, these findings suggest that active rTMS resulted in a significant potentiation in $\gamma$ power that was greatest in the N-back conditions with the greatest WM load. Figure 4A shows mean absolute change in $\gamma$ power as topographical illustrations (represented by hot colours). Inspection of the topographical plots revealed a maximal change in $\gamma$ power in the frontal brain regions compared to other cortical regions following active stimulation. As such, a repeated measures ANOVA was performed comparing the mean sum change in $\gamma$ power in 5 frontal electrodes (FPZ, FP1, FP2, AF3, and AF4) selected based on hot coloured electrodes from the topographical plot to 5 electrodes from the posterior region (OZ, O1, O2, PO3, and PO4) with rTMS as a between-subject factor. A significant effect of brain region was found $(F_{(1,19)}=14.938, p<0.001; \text{Figure 4B})$ and the brain region x rTMS interaction was also significant $(F_{(1,19)}=11.445, p<0.005)$. Finally, paired t-tests revealed that this interaction was due to the difference between brain regions following active stimulation $(t_{(10)} = 4.101, p < 0.005; \text{Figure 4B})$, while no difference between brain regions was found following sham stimulation $(t_{(9)} = 0.619, p > 0.05; \text{Figure 4B}; \text{SPSS 15.0, SPSS Inc. Chicago, Illinois, USA})$. These results further suggest that active rTMS resulted in a significant potentiation in $\gamma$ power isolated to the frontal brain regions during N-back conditions with the greatest WM load.
4.4.2 EEG Spectral Analysis of Other Frequency Bands

To test whether our findings of enhanced mean γ power following active rTMS elicited during the N-back task was selective to this frequency band, four separate MMRM analyses (active vs. sham) as a between-subject factor and time (pre vs. post), and WM load (0- versus 1- versus 2- versus 3-back) as within-subject factors were performed on the log transformed mean evoked δ (1-3.5 Hz), θ (4-8 Hz), α (9-12 Hz), and β (14-28 Hz) power for TC and NTC responses. An effect of WM load was revealed in all frequency bands; however, there were no differences between active and sham stimulation under any WM load. Finally, a time x group interaction was also revealed in the θ, and β frequency bands, but again with no difference between active and sham frequency power at either baseline (pre) or following rTMS (post). These subsequent spectral analyses, thus, demonstrate that active stimulation selectively enhanced the oscillatory activity in the γ band only, while the activity in the other frequencies remained unchanged.

4.4.3 N-Back Working Memory Performance

As expected, the MMRM analysis on WM performance revealed a significant effect of WM load \((F_{(2, 40)} = 78.63, p<0.001)\) with performance decreasing with increasing cognitive demand. In particular, subjects performed significantly better in the 1-back compared to the 2- \((p=0.0005)\) and 3-back \((p<0.0001)\) conditions and better in the 2-back relative to the 3-back \((p<0.0001)\) condition, respectively (Table 2). However, the interaction between time and group was not significant, reflecting similar WM performance across groups (i.e., active versus sham) pre and post-rTMS \((F_{(1, 20)} = 0.31, p=0.5859)\). Finally, no relationship was found between mean WM performance and mean γ power within any N-back condition determined through a Pearson correlation coefficient.
As expected, we found an effect of WM load on reaction time \( (F_{(3,60)} = 126.64, p<0.0001; \) Table 3) with reaction time increasing with increasing WM load. Specifically, pairwise comparisons revealed significantly lower RT in the 0-back relative to the 1-3-back conditions \( (p<0.0001 \) in each case), while reaction time was lower in the 1-back compared to the 2- \( (p=0.0001) \) and 3-back condition \( (p<0.0001) \), respectively.

### 4.5 Discussion

We measured the effect of 20 Hz rTMS applied to the DLPFC on γ oscillatory activity across WM load (i.e., 0-, 1-, and 2-back conditions). As predicted, γ oscillatory activity generally increased with increased WM load. Active rTMS significantly increased γ oscillatory activity compared to baseline (pre) and sham stimulation. Moreover, active rTMS caused the greatest change in γ oscillatory activity in the N-back conditions with the greatest cognitive demand, an effect that was limited to frontal brain regions. Finally, active rTMS had no effect on other frequency ranges (i.e., δ, θ, α, β) suggesting a selective effect to oscillatory activity in the γ frequency range. Collectively, these results suggest that active rTMS applied to bilateral DLPFC significantly increased frontal γ oscillatory activity which was most pronounced at N-back conditions of greatest difficulty.

The finding that γ oscillatory activity was most pronounced at N-back conditions of greatest difficulty is consistent with previous studies examining the role of γ oscillatory activity and WM (Howard, Rizzuto et al. 2003; Basar-Eroglu, Brand et al. 2007; Meltzer, Zaveri et al. 2008). For example, Howard and colleagues (2003) first demonstrated a linear increase in γ oscillatory power with WM load in epileptic patients performing the Sternberg WM task (Howard, Rizzuto et al. 2003). Additional studies confirmed enhanced γ oscillatory activity with WM load employing both the Sternberg (Meltzer, Zaveri et al. 2008) and N-back (Basar-Eroglu, Brand et
al. 2007) tasks. In these studies, however, WM loads comparable to the 3-back condition were not examined. In this regard, we observed significantly lower γ oscillatory activity in the 3-back condition relative to the 2-back condition at baseline in both groups ($p < 0.0004$). This finding is consistent with decreased BOLD activity elicited while healthy subjects performed the 3-back condition in fMRI studies. It has been suggested that these decreases in BOLD activity may be because WM resources are exceeded during the 3-back condition resulting in poorer cognitive performance due to diminished attentional resources (Cowan 2001; Kane, Bleckley et al. 2001; Wheeler and Treisman 2002). Though speculative, the fact that active rTMS to the DLPFC resulted in an increase in γ oscillatory activity, with the greatest effect in the 3-back condition (post; Figure 3A), suggests that rTMS, in part, may enhance attentional resources underlying such effects.

High-frequency rTMS has been shown to improve cognitive performance (Little, Kimbrell et al. 2000; Sparing, Mottaghy et al. 2001; Martis, Alam et al. 2003; O'Connor, Jerskey et al. 2005) though the underlying mechanisms have not been investigated. We contend that a possible explanation is through its effects on GABAergic inhibitory neurotransmission critical in both the generation and synchronization of oscillatory activity (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007). GABAergic neurons in cortical networks interact with other neurons to affect spike timing and coordinate rhythmic population activity (Mann and Paulsen 2007). GABAergic neurons have also been implicated in the synchronization of pyramidal neurons in the DLPFC during WM (Lewis, Hashimoto et al. 2005). For instance, Wilson and colleagues (1996) demonstrated that in monkeys, fast spiking GABAergic neurons in the DLPFC remain active during the delay period of WM tasks (Wilson, O'Scalaidhe et al. 1994). Additionally, during WM tasks, the activity of these GABAergic neurons contribute to
the spatial tuning of the neuronal response, and are thus considered to be related to the WM task itself (Rao, Williams et al. 2000), while the injection of GABAergic antagonists in the DLPFC resulted in a disruption in WM performance (Sawaguchi, Matsumura et al. 1989). Taken together with the finding that 20 Hz rTMS potentiates GABAergic inhibitory neurotransmission (Daskalakis, Moller et al. 2006), we contend that high-frequency rTMS exerts its effects on γ oscillatory activity via GABA receptor-mediated inhibitory neurotransmission to ultimately affect WM performance. Future studies combining paired pulse TMS with EEG recording to index GABA receptor-mediated neurotransmission in the DLPFC (Daskalakis, Farzan et al. 2008) will be needed, however, to determine if γ oscillatory activity is indeed potentiated directly through enhanced GABA.

Previous studies have also reported modulations in other frequency ranges during cognitive tasks. Although changes in oscillatory activity in the γ frequency band are most consistently observed, increases in the θ, α, and β frequency bands have also been associated with cognitive tasks (Gevins, Smith et al. 1997; Klimesch, Doppelmayr et al. 1997; Sarnthein, Petsche et al. 1998; Klimesch 1999; Tallon-Baudry, Kreiter et al. 1999; von Stein, Rappelsberger et al. 1999; Tesche and Karhu 2000; Klimesch, Doppelmayr et al. 2001; Raghavachari, Kahana et al. 2001; Jensen, Gelfand et al. 2002; Schack, Vath et al. 2002; Howard, Rizzuto et al. 2003; Rizzuto, Madsen et al. 2003; Gruber and Muller 2005; Kaiser and Lutzenberger 2005). In line with these studies, we found an increase in δ, θ, α, and β activity with WM load. However, following rTMS to the DLPFC, modulations were only found within the γ frequency range, while δ, θ, α, and β activities remained unchanged. These findings indicate that δ, θ, α, β, and γ activities are modulated by cognitive demand yet only γ activity was enhanced through rTMS over the DLPFC – a brain region that is closely associated with attention and WM performance.
To our knowledge, this is the first demonstration of enhanced γ oscillatory activity elicited during the N-back task following a single session of rTMS over the DLPFC. Although γ oscillatory activity was potentiated following rTMS, this effect was not related to WM performance. It may be possible that rTMS induced cognitive changes are either delayed or optimal at some later time point and/or that repeated sessions (i.e., days to weeks) of rTMS are needed for such effects to be fully realized. In this experiment we tested subjects in the N-back task within 20 minutes following rTMS administration, which may have been adequately captured neurophysiological but not the anticipated cognitive changes. This contention is consistent with literature from rTMS treatment studies. For example, in patients with major depressive disorder, high frequency 10 Hz rTMS applied to the left DLPFC two or three times per week (an average of 10.8 total treatments) was shown to improve cognitive performance and lessen the number of memory complaints approximately 8.8 days following the last administration of rTMS (Schultze-Rauchenbach, Harms et al. 2005). Similarly, patients with Parkinson’s disease with concurrent depression showed improvements in the Stroop and Harper & Wisconsin test performances 2- and 8 weeks following 10 sessions of 15 Hz rTMS applied to the left DLPFC (Boggio, Fregni et al. 2005). It has also been suggested that repeated sessions of rTMS may be necessary to produce changes in gene expression and synapse formation that accompany changes in short-term plasticity and cognition (Kheder, Rothwell et al. 2006). Further studies, therefore, are needed to determine if the anticipated changes to WM performance, that may follow enhanced γ oscillatory activity, are either delayed or require multiple rTMS sessions to be fully realized.

Oscillations in the γ frequency range are typically estimated using two sub-classifications that differ in their phase relationship with respect to the stimulus onset. Evoked γ oscillatory activity is phase-locked to the stimulus and occurs approximately 200 msec relative to stimulus onset.
Since evoked γ oscillatory activity is phase-locked to the stimulus, these responses can be extracted in a time domain and averaged across trials (Pantev 1995). In contrast, induced γ oscillatory activity is not phase-locked to the stimulus, but rather jitters in latency from trial and trial and, thus, cannot be averaged across trials. Induced γ oscillatory activity is measured by applying the time-frequency decomposition to each trial and the ensuing power is averaged across trials. The power of evoked and background components are then subtracted from the total power to provide an estimate of induced γ oscillatory activity (David, Kilner et al. 2006). Although there are numerous reports that support a relationship between induced γ oscillatory activity and cognitive functions (Gray, Konig et al. 1989; Fries, Reynolds et al. 2001; Pesaran, Pezaris et al. 2002; Cho, Konecky et al. 2006), a recent study demonstrated that measures of induced γ activity may not reflect synchronous neuronal activity, but rather the activation of miniature saccadic eye movements (Yuval-Greenberg, Tomer et al. 2008). In their study, Yuval-Greenberg et al (2008) recorded eye movements simultaneously with EEG and found that induced γ activity was time-locked to the onset and the rate of involuntary miniature saccades and thus, reflects saccadic spike potentials rather than neuronal oscillations. These authors contend that measures of evoked γ activity may better mitigate such effects from miniature saccadic activity on this neurophysiological phenomenon (Yuval-Greenberg, Tomer et al. 2008). Considering that induced γ activity may reflect involuntary eye movements with emerging evidence supporting the association of evoked γ activity with WM (Howard, Rizzuto et al. 2003; Basar-Eroglu, Brand et al. 2007; Meltzer, Zaveri et al. 2008), we selected to measure the effect of rTMS on evoked γ activity.

The results of this study are limited by the relatively small sample size, which may have attributed to the lack of a relationship found between increased gamma oscillatory activity and WM performance. Replication studies assessing the effect of rTMS on WM may consider using
a larger sample size to further examine this relationship. However, the fact that rTMS enhanced
γ oscillatory activity compared to sham, which was not observed within the other frequency
ranges, suggests that the observed potentiation of γ oscillatory activity was not related to the
small sample size. Nevertheless, such findings should be replicated in a larger sample to
minimize Type II error and stabilize statistical parameter estimates (Norman and Streiner 2000).

A second limitation to this study is the use of the N-back task to evaluate gamma oscillatory
activity underlying WM. Previous studies that have examined the convergent validity between
the N-back task with other WM measures such as the operation span task (OSPAN) show
correlations with lower WM loads (Shelton, Metzger et al. 2007) and varied results with the 3-
back condition (Kane, Conway et al. 2007; Shelton, Metzger et al. 2007). Such studies suggest
that at higher WM loads, attentional components and/or short-term memory processes may be
measured rather than WM capacity exclusively. Our finding of increased gamma oscillatory
activity following rTMS over the DLPFC may, therefore, reflect enhancements in attention
and/or short-term memory processes rather than WM capacity exclusively. Despite these
limitations, however, our findings provide a neurophysiological framework through which to
evaluate rTMS as a therapeutic tool in patient populations (e.g., schizophrenia) where WM
deficits form part of the symptom profile.

In summary, we demonstrated that high-frequency rTMS over the DLPFC significantly enhanced
frontal γ oscillatory elicited in N-back conditions with the greatest task difficulty. Furthermore,
rTMS administered to the DLPFC selectively increased oscillatory activity in the γ frequency
range, while other frequency ranges (i.e., δ, θ, α or β) remained unchanged. The specificity of
high-frequency rTMS on γ oscillatory activity found in this study may, therefore, provide
important insight into the pathophysiology of brain disorders that present cognitive deficits as part of their symptom profiles.
Table 1. Mean number of trials (TC + NTC) following artifact correction for each N-back condition pre and post rTMS administration.

<table>
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<tr>
<th>Group</th>
<th>Pre rTMS</th>
<th>Post rTMS</th>
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<tbody>
<tr>
<td></td>
<td>0-back</td>
<td>1-back</td>
</tr>
<tr>
<td>Sham</td>
<td>141.7</td>
<td>127.9</td>
</tr>
<tr>
<td>Active</td>
<td>127.9</td>
<td>137.6</td>
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Table 2. Performance across N-Back task condition for target and non-target correct responses expressed as percentage (%) before and after sham or active rTMS. Data expressed as mean (±) standard deviation (SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre rTMS</th>
<th>Post rTMS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1-back</td>
<td>2-back</td>
</tr>
<tr>
<td>Sham</td>
<td>92.1 (±)</td>
<td>2.3</td>
</tr>
<tr>
<td>(±) 1SD</td>
<td></td>
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<tr>
<td>Active</td>
<td>83.1 (±)</td>
<td>11.6</td>
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<tr>
<td>(±) 1SD</td>
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</tbody>
</table>
Table 3. Reaction time across N-Back task condition for target and non-target correct responses expressed in milliseconds (msec) before and after sham or active rTMS. Data expressed as mean (±) standard deviation (SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre rTMS</th>
<th>Post rTMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-back</td>
<td>1-back</td>
</tr>
<tr>
<td><strong>Sham (±)</strong></td>
<td>401.4 (±)</td>
<td>720.8 (+/-)</td>
</tr>
<tr>
<td>1SD</td>
<td>110.6</td>
<td>99.3</td>
</tr>
<tr>
<td><strong>Active (±)</strong></td>
<td>393.0 (±)</td>
<td>793.6 (±)</td>
</tr>
<tr>
<td>1SD</td>
<td>122.3</td>
<td>155.1</td>
</tr>
</tbody>
</table>
Figure 1. N-Back WM Task. A) A representation of the four different WM Conditions (0, 1, 2, and 3-back) that were administered in a randomized order to participants before and after rTMS. B) The timing of one trial from the presentation of a one letter separated by a (+) sign followed by a subsequent letter for a total time of 3000 msec. Participants were required to push one button (target) if the present letter was identical to the letter presented “N” trials back; otherwise the participants pushed a different button (non-target).
Figure 2. Targeting the Dorsolateral Prefrontal Cortex for rTMS Administration. Transverse view from a single subject with exposed cortex and overlap of Brodmann areas 9 & 46, highlighted (white) on a T1-weighted 3D MRI. Using MRI-to-MiniBird co-registration, the centre of the TMS coil was held over this region.
**Figure 3.** Mean log transformed change in oscillatory power (post rTMS frequency power-pre rTMS frequency power) following rTMS elicited during the N-back task for A) γ power compared to δ, θ, α, and β activities following B) sham, and C) active stimulation. Error bars represent (+) standard deviation (SD).
**Figure 4.** A) Topographical illustration of mean absolute change (post rTMS $\gamma$ power-pre rTMS $\gamma$ power) in $\gamma$ power elicited during 3-back condition following sham and active rTMS. Maximal change in $\gamma$ power (represented by hot colours) was found following active stimulation in the frontal brain regions. B) Change in the mean sum of $\gamma$ power in the frontal (FPZ+FP1+FP2+AF3+AF4) versus posterior (OZ+O1+O2+PO3+PO4) electrodes in the 3-back condition following sham and active stimulation. Error bars represent (+) standard deviation (SD).
5 Study 3: The Effect of Transcranial Magnetic Stimulation on Gamma Oscillatory Activity in Schizophrenia

5.1 Abstract

Gamma (γ) oscillations (30-50 Hz) have been shown to be excessive in patients with schizophrenia (SCZ) during working memory (WM). WM is a cognitive process that involves the online maintenance and manipulation of information that is mediated largely by the dorsolateral prefrontal cortex (DLPFC). Repetitive transcranial magnetic stimulation (rTMS) represents a non-invasive method to stimulate the cortex that has been shown to enhance cognition and γ oscillatory activity during WM. We examined the effect of 20 Hz rTMS over the DLPFC on γ oscillatory activity elicited during the N-back task in 24 patients with SCZ compared to 22 healthy subjects. Prior to rTMS, patients with SCZ elicited excessive γ oscillatory activity compared to healthy subjects across WM load. Active rTMS resulted in the inhibition of frontal γ oscillatory activity in patients with SCZ, while potentiating activity in healthy subjects in the 3-back, the most difficult condition. Further, these effects on γ oscillatory activity were found to be specific to the frontal brain region and were absent in the posterior brain region. We suggest that this opposing effect of rTMS on γ oscillatory activity in patients with SCZ versus healthy subjects may be related to homeostatic plasticity leading to differential effects of rTMS on γ oscillatory activity depending on baseline differences. These findings provide important insights into the neurophysiological mechanisms underlying WM and demonstrated that rTMS can inhibit excessive γ oscillatory activity in SCZ, a finding that may ultimately translate into a better understanding of the mechanisms leading to cognitive improvement.
5.2 Introduction

Gamma (\(\gamma\)) oscillations (30-50 Hz) are associated with working memory (WM). WM involves the maintenance and manipulation of information (Baddeley 1986) and has been shown to increase \(\gamma\) oscillations with increases in WM load in healthy subjects (Basar-Eroglu, Brand et al. 2007), particularly in the dorsolateral prefrontal cortex (DLPFC; (Barr, Farzan et al. 2009)). Schizophrenia (SCZ) patients have marked deficits in WM (Weinberger, Berman et al. 1986) that has been attributed to altered \(\gamma\) oscillatory activity. For example, we demonstrated that SCZ patients compared to healthy subjects elicit excessive \(\gamma\) oscillatory activity while performing the N-back task at all WM loads that was accompanied by impaired performance (Barr, Farzan et al. 2010). Although previous studies provide evidence for reduced \(\gamma\) oscillatory activity in SCZ patients during cognitive control (Cho, Konecky et al. 2006) and sensory oddball (Spencer, Niznikiewicz et al. 2008) tasks, recent findings suggest that during WM performance that \(\gamma\) oscillatory activity is excessive, even at equivalent performance levels (Basar-Eroglu, Brand et al. 2007; Barr, Farzan et al. 2010). Altered \(\gamma\) oscillatory activity in SCZ patients may therefore be related to impaired WM function in this disorder.

Animal studies have shown that \(\gamma\) oscillations during WM are supported by \(\gamma\)–aminobutyric acid (GABA) interneurons in the DLPFC (Sawaguchi, Matsumura et al. 1989; Wilson, O'Scalaidhe et al. 1994; Rao, Williams et al. 2000). Specifically, GABAergic activity may be involved in the generation and inhibition of \(\gamma\) oscillations (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007; Brown, Davies et al. 2007), a mechanism that has been shown to be impaired in SCZ (Benes, McSparren et al. 1991; Akbarian, Kim et al. 1995; Hashimoto, Volk et al. 2003; Lewis, Hashimoto et al. 2005). In line with these studies, Farzan et al. (2010) measured neurophysiological indices of GABA\(_B\) receptor inhibition from the DLPFC in SCZ.
patients compared to bipolar disordered patients and healthy subjects through combined TMS-EEG (Farzan, Barr et al. 2010). It was demonstrated that the inhibition of $\gamma$ oscillations was significantly reduced in DLPFC of SCZ patients compared to the other 2 groups (Farzan, Barr et al. 2010). It is possible, therefore, that deficits in the inhibition of $\gamma$ oscillations in the DLPFC in SCZ results in excessive $\gamma$ activity as was previously reported (Barr, Farzan et al. 2010), which may contribute to WM deficits in this disorder.

By contrast, in healthy subjects it was demonstrated that rTMS over the DLPFC selectively enhanced $\gamma$ oscillatory activity that was most pronounced in 3-back (Barr, Farzan et al. 2009), which may be related to its ability to its potentiating effects on GABAergic neurotransmission (Daskalakis, Moller et al. 2006; Jung, Shin et al. 2008). The aim of the current study was to examine the effect of rTMS over the DLPFC on $\gamma$ oscillatory activity during the N-back task in SCZ patients compared to healthy subjects. It was hypothesized that rTMS would inhibit excessive $\gamma$ oscillatory activity in SCZ patients compared to sham stimulation and in contrast to healthy subjects.

5.3 Materials and Methods

5.3.1 Subjects

Twenty-four (males=14; females=10) patients with a diagnosis of SCZ or schizoaffective disorder confirmed by the Structured Clinical Interview for DSM-IV (Spitzer 1994) and 22 (males=11; females=11) healthy individuals participated in this study. All subjects were right handed confirmed using the Oldfield Handedness Inventory (Oldfield 1971). Patients with SCZ were all treated with antipsychotic medication (14.4 ± 10.9 mg olanzapine, 6 patients; 233.3 ± 230.9 mg clozapine, 3 patients; 5.2 ± 3.0 mg risperidone, 7 patients; 733.3 ± 416.3 mg of quetiapine, 3 patients; 2.4 ± 1.6 mg fluphenazine, 3 patients; 25 mg haloperidol, 1 patient; 15
mg aripiprazole, 1 patient). Demographic data of the subject groups are shown in Table 1. The subject groups were similar in age \( t_{44} = -0.754, p=0.455 \), but differed in education (independent t-tests: \( t_{44} = 2.954, p<0.05 \); Table 1). Severity of psychopathology was evaluated using the positive and negative symptom scale (PANSS; (Kay, Fiszbein et al. 1987)), scale for the assessment of negative symptoms (SANS; (Andreasen 1989)) and the Calgary Depression Scale (CDS; (Addington, Addington et al. 1993); Table 1). Exclusion criteria for all subjects included a history of substance abuse or dependence in the last 6 months determined through the DSM-IV, a concomitant major and unstable medical or neurologic illness or pregnant. In healthy subjects the presence of psychopathology was ruled out through the personality assessment screener (PAS; Psychological Assessment Resources, Inc). Finally, all subjects provided their written informed consent and the protocol was approved by the Centre for Addiction and Mental Health in accordance with the declaration of Helsinki.

5.3.2 Procedure

This study was a randomized, double-blind, placebo-controlled design. Patients with SCZ and healthy subjects were randomized into two groups allocated to receive either active or sham rTMS. The experiment took place over two testing days. On the first day, subjects performed the N-back test while their EEG was recorded. One week later, rTMS was administered over the DLPFC followed by the final testing of the N-back task. The final N-back task was performed approximately 20 minutes following the rTMS administration to allow for cortical plasticity to take place as well as for the placement of the EEG cap. These two N-back testing sessions will be referred to ‘pre’ and ‘post’ measures relative to the rTMS administration here on in.
5.3.3 N-Back Task

Subjects performed the N-back task while their EEG was recorded (STIM2, Neuroscan, U.S.A.) pre and post rTMS. Stimuli were presented on a computer monitor one at a time and participants were required to push one button (target) if the present stimulus was identical to the stimulus presented “N” trials back; otherwise, subjects pushed a different button (non-target). Thus, the effect of increasing cognitive demand on oscillatory activity was tested by varying the “N” in the 1-, 2- and 3-back conditions. Stimuli consisted of black capital letters presented for 250 msec followed by a delay period of 3000 msec during which the subject was required to respond (Figure 1). In the 1- and 2-back conditions, stimuli were presented continuously for 15 minutes and for 30 minutes in the 3-back condition. The 3-back was administered for double the length of time to ensure a satisfactory number of correct responses were contained for the data analysis (Table 2). The number of target letters in each condition was: 46 of 198 (23.2%) 1-back; 31 of 197 trials (15.7%) 2-back, and 59 of 400 trials (14.6%) 3-back condition. The N-back task took 1 hour for subjects to complete with the order of conditions randomized and counterbalanced to prevent order effects.

5.3.4 Repetitive TMS

Repetitive TMS was administered using a Medtronic MagPro stimulator (Medtronic, Inc., U.S.A.) with a 70 mm diameter figure-of-8 coil to the right and left DLPFC at 20 Hz, 90 % resting motor threshold for 25 trains comprising of 30 pulses per train, inter-train interval of 30 seconds for a total of 750 pulses per hemisphere in accordance with published safety guidelines (Chen, Gerloff et al. 1997). The total time for the rTMS administration was 25 minutes, 12.5 minutes per hemisphere. The resting motor threshold was defined as the lowest intensity that produced a motor evoked potential of at least 50 µV in 50% of the trials delivered. Sham stimulation was delivered at the same rTMS parameters as active stimulation with the coil held
in a single wing-tilt position at 90 degrees to induce similar somatic sensations as in the active stimulation with minimal direct brain effects. The order of stimulation (right then left versus left then right) was also randomized and counterbalanced to prevent order effects.

5.3.5 DLPFC Site Localization

The localization of the DLPFC was determined through neuronavigational techniques using the MINIBIRD system (Ascension Technologies) combined with MRICro/Reg software using a T1-weighted MRI scan obtained for each subject with seven fiducial markers in place. Repetitive TMS was targeted at the junction of the middle and anterior one-third of the middle frontal gyrus (Talairach coordinates (x, y, z) = +/- 50, 30, 36) corresponding with posterior regions of Brodmann area 9 (BA9), and overlapping with the superior region of BA46 (Figure 2). The selection of this site was based on recent meta-analyses of functional imaging studies that examined WM and the activation of the DLPFC (Cannon, Glahn et al. 2005; Mendrek, Kiehl et al. 2005; Tan, Choo et al. 2005).

5.3.6 EEG Recording

EEG data were acquired using a 64-electrode cap and Synamps2 DC-coupled EEG system (Compumedics, U.S.A.). Four electrodes placed on the outer side of each eye, above, and below the left eye were used to monitor eye movement artifact. Data was recorded at a rate of 1000 Hz DC and with a 0.3 to 200 Hz band pass hardware filter. Electrode impedances were lowered to < 5 kΩ. All channels were referenced to an electrode placed posterior to the Cz electrode.

5.3.7 Offline EEG processing

We measured mean evoked oscillatory power over the delay period according to published protocol (Barr, Farzan et al. 2009). Data was filtered off-line using a 1 to 100 Hz band pass zero phase shift filter (slope, 24 dB/oct). Epochs were defined as –1000 to +3095 msec relative to the
cue onset and were baseline corrected with respect to the prestimulus interval (-1000 to cue onset). All trials were manually inspected and any error trials or epochs containing artifact (movement or electrooculogram exceeding +/- 50 µV) were excluded from further analysis.

5.3.8 Data Analysis

5.3.8.1 Behavioural Analysis

The total number of correct trials (target correct (TC) and non-target correct (NTC)) including those trials rejected due to artifact were included in the data analysis for WM performance and reaction time. Two separate mixed model repeated measures (MMRM) for WM and reaction were performed were performed on change score (post rTMS-pre rTMS) with Group (patients with SCZ versus healthy subjects) and rTMS (active versus sham) as between-subject factors and WM load (1- versus 2- versus 3-back) as the within-subject factor with a significance level set at p<0.05. Interaction effects were further examined with Bonferroni-adjusted pairwise comparisons (SAS System v.9.1.3; SAS Institute, NC, USA).

5.3.8.2 EEG Analysis

Artifact-free EEG data were imported into MATLAB (The MathWorks, Inc. Natick, MA, USA) using the EEGLAB toolbox (Delorme and Makeig 2004) for subsequent analysis. Evoked oscillatory power for each frequency (δ (1-3.5 Hz); θ (4-7 Hz); α (9-12 Hz); β (14-28 Hz) and γ, (30-50 Hz)) were averaged over the delay period (0-3000 msec from cue onset) for the target correct (TC) and non-target correct (NTC) responses for each WM load pre and post rTMS for each subject. Mean evoked oscillatory power was then assessed during these responses (TC and NTC) from the frontal electrodes (AF3, AF4, F5, F3, F1, FZ, F2, F4, and F6), and averaged for each subject. Since spectral analysis of EEG activity is often not normally distributed (Bender, Schultz et al. 1992), the data was log transformed prior to analysis. A series of seven (across
oscillatory power in the 5 frequency bands, N-back performance and reaction time) MMRM were performed on change score (post rTMS-pre rTMS) with Group (patients with SCZ versus healthy subjects) and rTMS (active versus sham) as between-subject factors and WM load (1-versus 2- versus 3-back) as the within-subject factor with a significance level set at p<0.05. The exclusion of the time (pre rTMS versus post rTMS) within subject factor was chosen to simplify the model to allow for an easier interpretation of a 3-way interaction versus a 4-way interaction term that would have been highly unstable. As such, the MMRM analyses were carried out on change scores for oscillatory power and behavioural data (post rTMS-pre rTMS). Interaction effects were further examined with Bonferroni-adjusted pairwise comparisons (SAS System v.9.1.3; SAS Institute, NC, USA).

5.4 Results

5.4.1 N-Back Behavioural Performance

Patients with SCZ performed worse than healthy subjects pre and post rTMS, however, there were no significant improvements observed in N-back performance following either active or sham rTMS stimulation in both subject groups found through the MMRM analysis (Table 3). Similarly, the MMRM analysis found no effect of rTMS on reaction time in both subject groups (Table 3).

5.4.2 Evoked γ Power

Prior to rTMS administration, excessive γ power was observed in patients with SCZ at each WM load compared to healthy subjects. The MMRM analysis on the change in mean γ power (post rTMS γ power-pre rTMS γ power) found a significant Group difference between patients with SCZ and healthy subjects ($F_{(1,42)}=18.23; p=0.0001$). Further, significant Group x rTMS ($F_{(1,42)}=10.37; p=0.0025$) and Group x N-back condition ($F_{(2,42)}=6.41; p=0.0037$) interaction
effects were found. The Group x rTMS x N-back interaction was also significant ($F_{(2,42)}=3.75$; $p=0.0317$; Figure 3A) indicating that the effects of Group and rTMS differed across WM load. A series of 15 Bonferroni-adjusted pairwise comparisons were then performed to better understand this 3-way interaction. Active stimulation was found to inhibit $\gamma$ power in patients with SCZ, while potentiating $\gamma$ power in healthy subjects. Moreover, this effect of active stimulation on $\gamma$ power differed significantly in patients with SCZ compared to healthy subjects in the 3-back condition ($p<0.0001$), while trending differences were observed in the 1- ($p=0.0750$) and 2-back ($p=0.0795$) conditions. To explore whether the effects of rTMS in the 3-back condition were specific to the frontal brain region, we compared mean $\gamma$ power from the frontal versus posterior brain region (electrodes: OZ, O1, O2, PO3, and PO4). A repeated measures ANOVA was conducted with Group, Time, and rTMS as between subject factors and brain region as a within subject factor (SPSS 15.0, SPSS Inc. Chicago, Illinois, USA) and a significant Time x Region x Subject x rTMS ($F_{(1,35)}=9.072$; $p=0.005$; Figure 3B) interaction was revealed. Pairwise comparisons found no differences in the posterior brain region in both subject groups following both active and sham stimulation. The effects of active rTMS on $\gamma$ oscillatory activity therefore were specific to the frontal brain region in the 3-back condition. These results suggest that active rTMS over the DLPFC inhibited frontal $\gamma$ power in patients with SCZ, while potentiating this activity in healthy subjects that was most pronounced in the 3-back condition with the greatest difficulty.

5.4.3 EEG Spectral Analysis of Other Frequency Bands

Although $\gamma$ oscillatory activity is most closely associated with higher order cognitive tasks, we explored the effect of rTMS on the mean change in oscillatory activities (post rTMS power-pre rTMS power) in the other frequency bands ($\delta$, $\theta$, $\alpha$, and $\beta$) with four separate MMRM analyses.
Although we observed a significant effect of Group on the change in mean oscillatory power in the $\theta$, $\alpha$, and $\beta$ frequency bands, no significant Group x rTMS interactions were observed (Figure 4). However, there was a significant Group x rTMS x N-back interaction found in the $\delta$ frequency band such that active rTMS reduced activity in patients with SCZ in the 3-back condition compared to sham stimulation ($p=0.0048$; Figure 4). No differences were found in healthy subjects in $\delta$ activation following rTMS administration.

5.4.4 Effect of Antipsychotic Medication

A Pearson correlation coefficient was performed to determine if the changes in $\gamma$ and $\delta$ oscillatory activity in the 3-back were related to antipsychotic medication using chlorpromazine equivalents (CPZ; (Woods 2003)) in the SCZ patient group. No relationships were found between $\gamma$ or $\delta$ oscillatory activities and CPZ equivalents in the 3-back pre or post rTMS administration.

5.5 Discussion

Our findings suggest that excessive frontal $\gamma$ oscillatory activity observed in SCZ patients was significantly inhibited following bilateral rTMS to DLPFC. By contrast, rTMS significantly potentiated $\gamma$ oscillatory activity in healthy subjects. These effects were most pronounced in the 3-back and were specific to the frontal cortical regions. rTMS also reduced $\delta$ activity in patients only. These results suggest that rTMS to DLPFC inhibits excessive frontal $\gamma$ oscillatory activity during the N-back in SCZ patients an effect that was opposite to that observed in healthy subjects.

The opposing effect of rTMS on $\gamma$ oscillatory activity in patients and healthy subjects may be related to differential changes in GABAergic activity. For example, Daskalakis et al. (2006) demonstrated that 20 Hz rTMS applied to the motor cortex in healthy subjects had different
effects depending on level of baseline GABAergic inhibitory neurotransmission. That is, rTMS potentiated short interval cortical inhibition (SICI), a neurophysiological paradigm that is related to GABA<sub>A</sub> receptor inhibition (Ziemann, Lonnecker et al. 1996), in subjects with relatively low baseline SICI and suppressed SICI in subjects with relatively high baseline activity (Daskalakis, Moller et al. 2006) suggesting that rTMS can produce variable effects on GABA<sub>A</sub> receptor mediated inhibition depending on baseline levels. As GABA<sub>A</sub> inhibitory post synaptic potentials are involved in generation of γ oscillations (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007) such findings can be used to explain the variable effects of rTMS on γ oscillatory activity in SCZ patients and healthy subjects. That is, rTMS inhibited γ oscillatory activity in SCZ patients with relatively greater γ activity at baseline, while potentiating activity in healthy subjects with relatively lower γ activity at baseline. Such effects may also be related to homeostatic plasticity, a brain mechanism that maintains neuronal activity within a useful physiological range and is critical to neuronal stability (Sejnowski 1977). There is considerable evidence for the role of GABA<sub>A</sub> receptor activity in the regulation of homeostatic plasticity (Hartmann, Bruehl et al. 2008; Le Roux, Amar et al. 2008; Wilhelm and Wenner 2008; Saliba, Gu et al. 2009; Chen, Shu et al. 2010) by modulating the number of post-synaptic GABA<sub>A</sub> receptors that are activated to increase or decrease inhibitory neurotransmission (Kilman, van Rossum et al. 2002; Swanwick, Murthy et al. 2006; Saliba, Michels et al. 2007; Hartmann, Bruehl et al. 2008). Regulation of GABA<sub>A</sub> receptors in homeostatic plasticity have also been shown to be involved in the synchronization of neuronal activity (Galarreta and Hestrin 1999; Gibson, Beierlein et al. 1999; Koos and Tepper 1999; Tamas, Buhl et al. 2000). The opposing effect of rTMS on γ oscillatory activity in the current study, therefore, may reflect differential regulation of inhibitory activity through efficacy of GABA<sub>A</sub> receptors important in homeostatic plasticity and generation of γ oscillations.
Alternatively, the effect of rTMS on γ oscillatory activity may reflect the regulation of cortical excitability to maintain homeostatic plasticity as GABA neurons are dependent on excitatory drive in generation of γ oscillations (Whittington, Traub et al. 1995; Traub, Whittington et al. 1996; Traub, Michelson-Law et al. 2004; Bartos, Vida et al. 2007; Mann and Paulsen 2007). In this regard, the main source of neuronal excitation is through release of glutamate which typically activates N-methyl-D-asparate (NMDA) and non-NMDA receptors in the post-synaptic membrane (Gonzalez-Burgos and Lewis 2008). The duration of non-NMDA excitatory post synaptic potentials (EPSPs) are optimal for fast signaling and coincidence detection. As such, non-NMDA EPSPs are important in the precise control of spike timing needed in the synchronization of cortical oscillations. The generation of oscillatory activity, therefore, may not only depend on GABA mediated inhibition but also on the recruitment of interneuron firing by glutamate excitation (Gonzalez-Burgos and Lewis 2008). Homeostatic plasticity has been shown through the alteration of cortical excitability with transcranial direct current stimulation (tDCS) and rTMS administered to the motor cortex in healthy subjects (Siebner, Lang et al. 2004). In their study, cathodal tDCS reduced corticospinal excitability followed by 1 Hz rTMS that resulted in a sustained increase in corticospinal excitability. By contrast, increased corticospinal excitability by anodal tDCS was subsequently reduced with 1 Hz rTMS. Those subjects with the greatest changes induced by tDCS priming also exhibited the greatest change in corticospinal excitability following rTMS (Siebner, Lang et al. 2004). Siebner et al. (2004) therefore demonstrate that rTMS can produce variable effects on cortical excitability depending on baseline activity level. Given the importance of excitatory drive on γ oscillations, the homeostatic regulation of cortical excitability through rTMS may also have produced our finding of opposing effects on γ oscillatory in patients versus healthy subjects.
As previously shown, rTMS had no effect on the δ, θ, α, and β frequency bands in healthy subjects (Barr, Farzan et al. 2009). In SCZ patients, however, rTMS reduced δ oscillatory activity in the 3-back compared to sham stimulation. This finding is consistent with a previous rTMS study in patients with SCZ with predominant negative symptoms (Jandl, Bittner et al. 2005). In this study, when rTMS was applied at 10 Hz to the left DLPFC for 5 days a reduction in negative symptoms and δ oscillatory activity was reported (Jandl, Bittner et al. 2005). It is possible that a reduced δ activity following rTMS in SCZ patients may be related to inhibition of γ oscillatory activity given the non-random relationships between oscillatory frequencies (Roopun, Kramer et al. 2008). That is, cross-frequency interactions or “nesting” is observed when the power of a discrete frequency band is modified by the phase of a lower frequency band that coexists during information processing (Roopun, Kramer et al. 2008). In this regard, hierarchies of nested rhythms have been observed in the neocortex (Penttonen and Buzsaki 2003) between δ, θ, and γ oscillatory activities. These findings suggest that γ oscillations are nested within δ oscillations and in relation to our findings, the effect of rTMS on γ activity in SCZ patients may, in part, be related to an altered δ oscillatory activity.

This study is limited in some important ways. First, although rTMS inhibited excessive γ oscillatory activity in SCZ patients and potentiated activity in healthy subjects, this change was not related to improved WM performance. Previous studies, however, have shown that rTMS induced cognitive changes are either delayed or optimal at some later time point (Boggio, Fregni et al. 2005; Schulze-Rauschenbach, Harms et al. 2005) and that repeated rTMS sessions may be needed to produce changes in gene expression and synapse formation associated with changes in short-term plasticity and cognition (Khedr, Rothwell et al. 2006). Nevertheless, this study provides early and interesting neurophysiological evidence for the modulating effect of rTMS on
γ oscillatory activity, a finding that warrants further investigation as a potential therapeutic mechanism which underlies WM impairments in SCZ. Second, the relatively small sample size tested may be insufficient to detect an improvement in performance on the N-back following rTMS. Replication studies may consider using a larger sample size to examine this relationship. However, the fact that rTMS altered γ oscillatory activity compared to sham, suggests that the change in γ was not related to the small sample size. Nevertheless, such findings should be replicated in a larger sample to minimize Type II error and stabilize statistical parameter estimates (Norman and Streiner 2000). Third, our finding of reduced γ oscillatory activity following rTMS may be related to the effect of antipsychotic medication. In this regard, Hong et al. (2004) reported enhanced 40 Hz oscillations in SCZ patients that were treated with second generation compared those taking conventional antipsychotics (Hong, Summerfelt et al. 2004). In this study, oscillations were parsed into 20, 30 and 40 Hz; however, 30-50 Hz range is most conventionally examined during cognitive tasks (Howard, Rizzuto et al. 2003). Similarly, a differential effect of antipsychotic medication on cognitive performance has also been reported with SCZ patients on second generation performing better than those patients on conventional antipsychotics on a variety of cognitive tests, including WM (Meltzer and McGurk 1999; Bilder, Goldman et al. 2002; Sharma, Hughes et al. 2003). In our sample, only 4 subjects were on conventional antipsychotics and these subjects happened to be randomly assigned to the sham group. Nevertheless, there were no differences found in γ oscillatory activity or in WM performance pre or post sham rTMS in those patients on conventional versus second generation antipsychotics. We were unable therefore to evaluate the effect of rTMS on γ oscillatory activity in SCZ patients on conventional versus second generation antipsychotics.

In summary, we demonstrated that rTMS over DLPFC alters frontal γ oscillatory activity with the greatest effect at higher WM loads. In patients, rTMS inhibited excessive γ oscillatory
activity across WM load. In contrast, rTMS potentiated $\gamma$ oscillatory activity in healthy subjects. The differential effect of rTMS on $\gamma$ oscillatory activity may be related to the concept of homeostatic plasticity involving the regulation of GABAergic inhibitory mechanisms that maintain neuronal excitability within a useful physiological range. These findings provide important insights into the neurophysiological mechanisms that may lead to cognitive potentiation in this disorder.
Table 1. Demographic Data for Healthy Subjects (HS) and patients with schizophrenia (SCZ) and the assessment of psychotic symptoms in patients with SCZ rTMS (±) 1 standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>HS</th>
<th>SCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>44.500 (±) 11.43</td>
<td>47.21 (±) 12.80</td>
</tr>
<tr>
<td>Age Range</td>
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<td>23-70</td>
</tr>
<tr>
<td>Female (n)</td>
<td>11</td>
<td>10</td>
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<tr>
<td>Male (n)</td>
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<tr>
<td>PANSS Scores</td>
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<tr>
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<td>17.50 (±) 6.40</td>
</tr>
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<td>Total</td>
<td>NA</td>
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<td>Psyrats Score</td>
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<td>Total</td>
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<td>37.67 (±) 21.42</td>
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Table 2. Total number (TC+NTC) of trials analyzed for healthy subjects (HS) and patients with schizophrenia (SCZ) in the 1-, 2-, and 3-back task conditions pre- post-rTMS (±) 1 standard deviation.

<table>
<thead>
<tr>
<th>No of Trials</th>
<th>Condition</th>
<th>HS</th>
<th></th>
<th></th>
<th>SCZ</th>
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<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
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<td></td>
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<td>Active</td>
<td>Sham</td>
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<td>Sham</td>
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<td>Sham</td>
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<tr>
<td>1-Back</td>
<td></td>
<td>130.91</td>
<td>130.00</td>
<td>127.09</td>
<td>114.81</td>
<td>82.00</td>
<td>84.17</td>
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<tr>
<td>(±) SD</td>
<td></td>
<td>39.77</td>
<td>24.61</td>
<td>42.37</td>
<td>48.82</td>
<td>42.21</td>
<td>43.79</td>
</tr>
<tr>
<td>2-Back</td>
<td></td>
<td>150.00</td>
<td>130.36</td>
<td>139.82</td>
<td>104.91</td>
<td>101.92</td>
<td>96.08</td>
</tr>
<tr>
<td>(±) SD</td>
<td></td>
<td>32.75</td>
<td>33.14</td>
<td>43.21</td>
<td>26.36</td>
<td>42.39</td>
<td>44.07</td>
</tr>
<tr>
<td>3-Back</td>
<td></td>
<td>283.82</td>
<td>225.64</td>
<td>264.45</td>
<td>181.64</td>
<td>157.27</td>
<td>168.42</td>
</tr>
<tr>
<td>(±) SD</td>
<td></td>
<td>76.22</td>
<td>62.92</td>
<td>79.65</td>
<td>77.80</td>
<td>73.38</td>
<td>76.71</td>
</tr>
</tbody>
</table>
Table 3. Working memory (WM) behavioural performance (%) and reaction time (RT; msec) during the N-back in healthy subjects (HS) versus patients with schizophrenia (SCZ) pre-post either active or sham rTMS (±) 1 standard deviation pre-post rTMS.

<table>
<thead>
<tr>
<th>Behavioural Condition</th>
<th>HS</th>
<th>SCZ</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td></td>
<td>Active</td>
<td>Sham</td>
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<tr>
<td>Score (%) 1-Back</td>
<td>83.19</td>
<td>92.12</td>
</tr>
<tr>
<td>(±) SD</td>
<td>11.61</td>
<td>2.26</td>
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<tr>
<td>2-Back</td>
<td>74.18</td>
<td>88.03</td>
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<tr>
<td>(±) SD</td>
<td>16.29</td>
<td>13.57</td>
</tr>
<tr>
<td>3-Back</td>
<td>66.86</td>
<td>77.41</td>
</tr>
<tr>
<td>(±) SD</td>
<td>11.58</td>
<td>15.39</td>
</tr>
<tr>
<td>RT (msec) 1-Back</td>
<td>793.56</td>
<td>720.78</td>
</tr>
<tr>
<td>(±) SD</td>
<td>147.18</td>
<td>99.26</td>
</tr>
<tr>
<td>2-Back</td>
<td>924.42</td>
<td>891.49</td>
</tr>
<tr>
<td>(±) SD</td>
<td>257.44</td>
<td>272.74</td>
</tr>
<tr>
<td>3-Back</td>
<td>990.42</td>
<td>970.08</td>
</tr>
<tr>
<td>(±) SD</td>
<td>317.13</td>
<td>247.70</td>
</tr>
</tbody>
</table>
Figure 1. A) A representation of the 1-, 2- and 3-back conditions that were completed in a randomized order by patients with schizophrenia (SCZ) and healthy subjects (HS) pre-post rTMS. Subjects were required to push one button (target) if the current letter was identical to the letter presented “N” trials back; otherwise the participants pushed a different button (non-target). Correct responses for target (TC) and non-target (NTC) were included in the data analysis. B) The timing of one trial from the presentation of a one letter separated by a (+) sign followed by a subsequent letter for a total time of 3000 msec.
Figure 2. Targeting the Dorsolateral Prefrontal Cortex (DLPFC) for rTMS stimulation. Transverse view from a single subject with exposed cortex and overlap of Brodmann areas 9 & 46, highlighted (white) on a T1-weighted 3D MRI. Using MRI-to-MiniBird co-registration, the centre of the TMS coil was held over this region.
Figure 3. A) Mean log transformed gamma oscillatory power (γ; 30-50 Hz; uV²) for target correct (TC) and non-target correct (NTC) responses during the N-back task pre-post rTMS in healthy subjects (HS; N=22) versus patients with schizophrenia (SCZ; N=24). B) Mean log transformed γ oscillatory power (uV²) for target correct (TC) and non-target correct (NTC) responses during the 3-back condition measured from the frontal and posterior brain regions pre-post rTMS in patients with schizophrenia (SCZ; N=24) and healthy subjects (HS; N=22). Bars represent (±) 1 standard deviation.
Figure 4. Mean log transformed oscillatory power (uV²) for target correct (TC) and non-target correct (NTC) responses during the N-back task pre-post rTMS in healthy subjects (HS; N=22) versus patients with schizophrenia (SCZ; N=24) across delta (δ; 1-3.5 Hz), theta (θ; 4-7 Hz), alpha (α; 8-12 Hz), and beta (β; 12.5-28 Hz) frequency ranges. Bars represent (±) 1 standard deviation.
6 Discussion

6.1 Summary of Results

The results presented in the previous chapters consist of three main findings: (a) patients with schizophrenia compared to healthy subjects generate excessive frontal evoked gamma oscillatory activity during the N-back task regardless of working memory load and at equivalent performance levels; (b) in healthy subjects, rTMS applied to the DLPFC selectively potentiates frontal evoked gamma oscillatory activity during a subsequent testing on the N-back task; and (c) rTMS over the DLPFC inhibits excessive frontal evoked gamma oscillatory in patients with schizophrenia in contrast to healthy subjects.

Study one measured frontal evoked oscillatory activity while performing the N-back working memory task in patients with schizophrenia compared to healthy subjects based on our a priori hypothesis that patients would exhibit an alteration in the gamma frequency band. We demonstrated two main group differences. First, patients with schizophrenia generated excessive frontal evoked gamma oscillatory activity compared to healthy subjects regardless of working memory load and at equivalent performance levels. Second, we observed reduced frontal evoked beta oscillatory at each working memory load in patients with schizophrenia compared to the healthy subject group. Furthermore, this study explored the relationship between frontal evoked oscillatory activity generated during the 3-back, working memory performance and symptom severity. We observed an inverse relationship between working memory performance and the severity of negative but not positive symptoms. The findings of this study, therefore, demonstrate that patients with schizophrenia exhibit impaired frontal evoked gamma oscillatory activity while performing the N-back task and raised two important questions. First, can high frequency rTMS applied to the DLPFC normalize excessive frontal evoked gamma oscillatory activity generated
during the N-back task in patients with schizophrenia? Second, if rTMS modulates frontal evoked gamma oscillatory activity, would these neurophysiological changes be related to improved performance on the N-back task? To answer these two questions, we administered high frequency rTMS to the DLPFC first in healthy subjects in study two and patients with schizophrenia in study three.

In the second study, we administered one session of 20 Hz rTMS applied bilaterally to the DLPFC to evaluate its effects on frontal evoked gamma oscillatory activity in healthy subjects on a subsequent testing on the N-back task. We hypothesized that rTMS would enhance frontal evoked gamma oscillatory activity based on previous studies which have shown that rTMS enhances oscillatory activity (Okamura, Jing et al. 2001; Griskova, Ruksenas et al. 2007) and GABAergic activity (Daskalakis, Moller et al. 2006) which have been shown to underlie the generation and modulation of gamma oscillations (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007). It was shown that rTMS applied to the DLPFC potentiated frontal evoked gamma oscillatory that was most pronounced in the more difficult working memory loads (i.e., 2- and 3-back conditions) compared to baseline activity and sham stimulation. Furthermore, we showed that this effect was isolated to the frontal brain region and was specific to activity in the gamma frequency range. This study demonstrated the ability of rTMS applied over the DLPFC to selectively potentiate frontal evoked gamma oscillatory activity in healthy subjects and raised one important question. Can excessive activity in patients with schizophrenia observed in study 1 be normalized by rTMS and improve working memory performance?

Study three administered 20 Hz rTMS applied bilaterally to the DLPFC to measure its effect on frontal evoked gamma oscillatory activity elicited during the N-back task in patients with
schizophrenia compared to healthy subjects. It was hypothesized that rTMS would inhibit excessive frontal evoked gamma oscillations in patients with schizophrenia compared to sham stimulation and in contrast to healthy subjects. It was demonstrated that rTMS over the DLPFC resulted in the inhibition of frontal evoked gamma oscillatory activity that was most pronounced in the 3-back condition. By contrast, rTMS potentiated frontal evoked gamma oscillatory activity in healthy subjects thereby replicating our finding from study two. We suggested that the differential effect of rTMS on frontal gamma oscillatory activity was due to differences in baseline activity levels in patients with schizophrenia compared to healthy subjects, a concept that may be related to homeostatic plasticity. Such homeostatic effects may be mediated through GABAergic inhibitory neurotransmission given its important role in both the generation and modulation of gamma oscillatory activity.

6.2 The Effect of rTMS on Frontal Evoked Gamma Oscillatory Activity and the Concept of Homeostatic Plasticity

The role of GABAergic inhibitory neurotransmission in the generation and modulation of gamma oscillations has been emphasized in this thesis. However, the excitatory drive mediated by glutamate to GABA neurons has also been shown to be critical in the generation of gamma oscillations (Whittington, Traub et al. 1995; Traub, Whittington et al. 1996; Traub, Michelson-Law et al. 2004; Bartos, Vida et al. 2007; Mann and Paulsen 2007). The balance or homeostasis between excitatory and inhibitory neurotransmission therefore is important in the regulation of gamma oscillations. Unlike slow-onset homeostasis that is involved in the adaptation to one’s environment, fast-onset homeostatic regulation is needed to quickly counteract any destabilization in function of the neuronal network (Chen, Chen et al. 2008). That is, the functional state of most central neuronal networks is highly dynamic which causes rapid changes in neuronal firing patterns, overall activity, and synchrony (Buzsaki and Draguhn 2004). In
regards to cortical oscillations, the pattern of neuronal activity depends not only on the architecture of the neuron, but also on its initial conditions (Glass 2001; Penttonen and Buzsaki 2003). That is, unless an oscillator is perturbed, the pattern of neuronal activity will repeat indefinitely in a noise-free system (Lisman and Idiart 1995). In relation to the findings of study three, the effect of rTMS on gamma oscillatory activity may have perturbed these oscillations which may have resulted in homeostatic mechanisms to regulate this activity based on initial baseline levels. That is, in patients with schizophrenia with initial excessive gamma oscillatory activity, rTMS resulted in the inhibition of this activity while potentiating this activity in healthy subjects with relatively lower baseline levels. This suggestion is consistent with previous rTMS studies. For example, rTMS has been shown to produce variable effects on inhibitory (Bagnato, Curra et al. 2005; Daskalakis, Moller et al. 2006) and excitatory (Siebner, Lang et al. 2004) mechanisms that were dependent on the level of baseline activity thereby demonstrating homeostatic mechanisms through rTMS.

Homeostatic plasticity is an important brain mechanism that maintains neuronal activity within a useful physiological range and is critical to neuronal stability and survival (Sejnowski 1977). For proper information processing, the level of activity in neuronal networks has to be maintained within a physiological range between absolute silence and over-excitation that occurs during seizures (Hartmann, Bruehl et al. 2008). In patients with schizophrenia, excessive gamma oscillatory activity was accompanied by deficits in working memory performance. Such excessive activity may reflect a dysregulation of homeostatic mechanisms as neural plasticity has been shown to be altered in patients with schizophrenia (Daskalakis, Christensen et al. 2008). Although, the administration of rTMS resulted in the inhibition of these oscillations, this neurophysiological change was not associated with improved working memory performance. Furthermore, oscillatory activity was measured within 20 minutes following rTMS stimulation...
and it is possible that gamma oscillatory activity may have returned to baseline levels following the testing session. In this regard, it has been suggested that repeated sessions of rTMS are needed produce changes in gene expression and synapse formation associated with changes in short-term plasticity and cognition (Khedr, Rothwell et al. 2006). By extension, repeated administration of rTMS may produce long lasting changes in oscillatory activity that may ultimately translate into improved working memory performance. Nevertheless, study three demonstrated that one session of rTMS may have perturbed gamma oscillatory activity resulting in variable effects on this activity in patients with schizophrenia and healthy subjects based on baseline activity. This finding may therefore reflect homeostatic mechanisms in the regulation of the excitatory and inhibitory balance important in gamma oscillations.

### 6.3 Is there an Optimal Level of Gamma Oscillatory Activity in Working Memory?

In the previous section, it was suggested that rTMS may serve to maintain frontal evoked gamma oscillatory activity within a useful physiological range. As described in chapter 1, oscillatory activity may be involved in the temporal framework for information encoding (Singer 1999; Fries, Nikolic et al. 2007) and that the interaction between activities in the various frequency bands may provide the brain with multiple times scales (Buzsaki 2006). In regards to working memory, it has been shown that gamma activity increases in response to increases in working memory load (Howard, Rizzuto et al. 2003) which has led to the hypothesis that gamma activity is used to organize and temporally segment the representations of different items in a multi-item working memory system (Lisman and Idiart 1995; Tallon-Baudry, Bertrand et al. 1996; Luck and Vogel 1997; Jensen and Lisman 1998; Pesaran, Pezaris et al. 2002). In line with this hypothesis, the series of studies presented in this thesis demonstrated that frontal evoked gamma oscillatory activity in the DLPFC increases in response to working memory load from the 0- to
the 2-back condition followed by a decrease in activity in the 3-back condition in healthy subjects resulting in an inverted U-shaped pattern. This inverted U-shaped pattern has also been reflected by the BOLD signal measured in an fMRI studies in healthy subjects performing the N-back task (Callicott, Mattay et al. 1999) that has been suggested to result from diminished attentional resources in the 3-back condition (Cowan 2001; Kane, Bleckley et al. 2001; Wheeler and Treisman 2002). The increase in frontal evoked gamma oscillatory activity following rTMS in healthy subjects shown in studies two and three may have been attributed to increased attention that is a critical component of working memory particularly during higher working memory loads (Shelton, Metzger et al. 2007). In this regard, attention has also been shown to modulate with gamma oscillatory activity (Brovelli, Lachaux et al. 2005; Vidal, Chaumon et al. 2006) and may reflect what aspect of working memory that was potentiated through rTMS resulting in a more linear pattern of frontal evoked gamma oscillatory activity in response to working memory load. In healthy subjects, therefore, increased gamma oscillatory activity appears to be beneficial while performing working memory tasks especially during higher working memory loads resulting in a more linear pattern of gamma activity in response to working memory load.

In patients with schizophrenia, the findings of studies one and three revealed that patients with schizophrenia generated excessive and similar frontal evoked gamma oscillatory activity across working memory load in contrast to the inverted U-shaped pattern observed in healthy subjects. Heightened levels of activity regardless of task demand has also been shown by Basar-Eroglu et al 2007 with EEG (Basar-Eroglu, Brand et al. 2007) and also with fMRI (Callicott, Mattay et al. 2003). Although from previous studies and the healthy subject findings presented in this thesis, it is hypothesized that greater gamma activity in response to working memory load is advantageous; however, too much activity does not optimize performance on these tasks at least
in patients with schizophrenia. This raises the following questions: is there an optimal level of gamma for working memory performance and is this level of gamma different for patients with schizophrenia and healthy subjects? The fact that increased frontal evoked gamma oscillatory activity was accompanied by impaired working memory performance in patients with schizophrenia compared to healthy subjects observed in studies one and three suggests that too much gamma is not advantageous in this disorder. In healthy subjects, however, study three showed that rTMS potentiated frontal evoked gamma oscillatory to a similar baseline level of gamma in patients with schizophrenia in the 3-back condition. It is therefore possible that rTMS may have had a deleterious effect on gamma activity in healthy subjects in generating too much gamma however we are unable to evaluate this possibility as there was no change in behavioural performance in healthy subjects following rTMS. Future studies are needed to investigate if there is an optimal level of gamma activity needed for working memory and if this level of gamma is different in patients with schizophrenia compared to healthy subjects. Alternatively, it is possible that the modulation of gamma in response to working memory load is critical to optimal working memory function rather than the amount of gamma generated. This possibility will be discussed in the following section.

6.4 The Modulation of Gamma Activity in Working Memory Through Cortical Inhibition

The results from studies one and three demonstrated that patients with schizophrenia fail to modulate frontal evoked gamma activity in response to increased working memory load. This finding is consistent with previous EEG studies testing working memory (Basar-Eroglu, Brand et al. 2007) and cognitive control (Cho, Konecky et al. 2006) and also with fMRI (Snitz, MacDonald et al. 2005). Taken together, these studies suggest that the lack of modulating gamma activity in response to working memory load may contribute to working memory deficits
in patients with schizophrenia. As previously described, cortical inhibition plays a critical role in gamma oscillations. Specifically it has been shown that GABA\textsubscript{A} receptor mediated IPSPs are involved in the generation of gamma oscillations (Whittington, Traub et al. 1995; Wang and Buzsaki 1996; Bartos, Vida et al. 2007), while GABA\textsubscript{B} receptor activity is involved in the modulation of gamma oscillations (Whittington, Traub et al. 1995; Brown, Davies et al. 2007; Leung and Shen 2007). In studies one and three, we demonstrated that patients with schizophrenia elicit excessive frontal evoked gamma oscillatory activity compared to healthy subjects regardless of working memory load and at equivalent performance levels. This finding suggests that in patients with schizophrenia there is impairment in the modulation of gamma oscillatory activity mediated by GABA\textsubscript{B} receptor activity rather than a deficit in its generation. In this regard, our lab has recently measured neurophysiological indexes of GABA\textsubscript{B} receptor mediated inhibition from the DLPFC in patients with schizophrenia compared to patients with bipolar disorder and healthy subjects through combined TMS-EEG (Farzan, Barr et al. 2010). It was demonstrated that the inhibition of gamma oscillations were significantly reduced in the DLPFC in patients with schizophrenia compared to the other two groups (Farzan, Barr et al. 2010). It is therefore possible that the lack of inhibition of gamma oscillations in schizophrenia may be related to our finding of excessive frontal evoked gamma oscillatory activity generated during the N-back task observed in study one and prior to rTMS administration in study three. It follows that the inhibition of frontal evoked gamma oscillatory activity following rTMS in patients with schizophrenia may have enhanced the activity of GABA\textsubscript{B} receptor mediated inhibitory neurotransmission. In this regard, Daskalakis et al (2006) demonstrated that the application of a single session of 20 Hz rTMS to the motor cortex resulted in the significant enhancement of a TMS neurophysiological index of GABA\textsubscript{B} receptor mediated inhibition in healthy subjects (Daskalakis, Moller et al. 2006). Together these studies suggest that the lack of
gamma modulation in patients may contribute to the deficits observed in working memory and that rTMS may have exerted its effect on frontal evoked gamma oscillatory activity through GABAergic inhibitory mechanisms.

6.5 Medication Effects on Cortical Excitability and Oscillatory Activity

As previously discussed, gamma oscillations are dependent on excitatory and inhibitory neurotransmission. Further, it has been demonstrated that antipsychotic medications influence excitatory and inhibitory activity in patients with schizophrenia however these findings have produced inconsistent findings. For example, Pascual-Leone et al (2002) reported increased motor threshold and decreased cortical inhibition in patients with schizophrenia on conventional antipsychotic medication, while unmedicated patients did not exhibit such differences compared to healthy subjects (Pascual-Leone, Manoach et al. 2002). By contrast, Daskalakis et al (2002) reported significant cortical inhibition deficits in unmedicated patients with schizophrenia, whereas no differences were found between medicated (conventional and unconventional antipsychotics) patients compared to healthy subjects (Daskalakis, Christensen et al. 2002).

Despite these divergent findings, deficits in cortical inhibition is a robust finding in patients with schizophrenia and has been suggested to result from: (1) excessive subcortical dopaminergic activity that results in either the disinhibition of cortical inhibitory neurotransmission (Walker 1994) or decreased activation of cortical inhibitory projections (Swerdlow and Koob 1987); and (2) reduced GABAergic interneurons in the prefrontal, anterior cingulate, and hippocampal formation (Benes, McSparren et al. 1991; Benes 1999). It has further been demonstrated that unconventional antipsychotic medications have different effects on cortical inhibition. For example, three studies conducted by the Daskalakis group indicate differences in cortical inhibition between patients treated with clozapine, olanzapine, and risperidone (Fitzgerald,
Brown et al. 2002; Daskalakis, Christensen et al. 2008; Liu, Fitzgerald et al. 2009). It was suggested that such medications may differ in their action on GABA, glutamate or through the modulation of ascending amine systems. The interaction of ascending dopaminergic and serotonergic pathways and cortical networks with both excitatory and inhibitory cortical connections is however still not fully understood. Taken together, these studies suggest that medication influences both cortical excitatory and inhibitory mechanisms in patients with schizophrenia through the interaction of several neurotransmitters which may in turn result in differential effects on gamma oscillatory activity.

As previously described, GABA plays a critical role in the generation and modulation of gamma oscillations. Antipsychotic medication that exerts its influence on GABAergic inhibitory neurotransmission may therefore differentially effect gamma oscillations compared to other antipsychotics. For example, clozapine may result in the blockade of GABA_A (Squires and Saederup 2000) and has been shown to increase GABA_B receptor activity (Daskalakis, Christensen et al. 2008) may modulate gamma oscillations in patients with schizophrenia. Alternatively, the blockade of GABA_A receptors by clozapine has been shown to increase the amplitude of theta and gamma oscillations (Squires and Saederup 2000) possibly through basket cells which can synchronize these frequencies in pyramidal cells by inducing rebound action potentials following GABAergic hyperpolarizing IPSPs (Cobb, Buhl et al. 1995). In addition, when GABA binds to GABA_B receptors, the release of dopamine, noradrenergic, and serotonin are inhibited (von Bohlen 2006) thus the action of clozapine on these other neurotransmitter systems may also influence gamma oscillations in patients with schizophrenia. In this regard, clozapine also interrupts the binding of dopamine at D_1, D_2, D_3, and D_5 receptors (Naheed and Green 2001), which may have resulted in excessive gamma oscillatory activity. For example, it has been shown in non-human primates that the blockade of D_1 and D_5 receptors during a
working memory task enhances task-related excitability (Williams and Goldman-Rakic 1995). This finding therefore suggests that clozapine’s antagonism of dopamine receptors may result in the excessive gamma oscillatory activity observed in patients with schizophrenia in studies one and three.

The effect of antipsychotic medications on lower frequencies in schizophrenia animal models has also been examined. For example, alteration of low frequency oscillatory activity by 5-HT$_{2A/2C}$ agonist DOI and noncompetitive NMDA-R antagonist phencyclidine (PCP) have been shown to reduce activity in the 3-4 Hz range that was subsequently reversed by application of both haloperidol and clozapine (Kargieman, Santana et al. 2007; Celada, Puig et al. 2008). Another study which examined the effect of acute haloperidol and chronic risperidone treatment on resting, evoked and induced oscillatory activities in a mice schizophrenia model reported that antipsychotic medications reduced resting theta and increased evoked gamma oscillatory activity during an auditory task. Similar to clozapine, therefore, haloperidol and risperidone treatment may have contributed to excessive gamma oscillatory activity in patients with schizophrenia demonstrated in studies one and three.

Taken together, these studies reveal differential effects of antipsychotic medications on cortical inhibition in patients with schizophrenia and on oscillatory activities with the use of schizophrenia animal models. Such development of animal models with altered EEG spectra can evaluate the contributions of GABA, dopaminergic and glutamatergic states in schizophrenia and also evaluate the confounding effects of antipsychotic medication on human EEG studies. Although an effect of medication was not found in studies one and three, these studies reviewed above suggest that antipsychotic medication that block GABA$_A$ or dopamine receptors may contribute to the excessive gamma oscillatory activity found in patients with schizophrenia.
6.6 Hypo or Hyperfunctioning of the Prefrontal Cortex Contribute to Cognitive Deficits?

There is considerable evidence for abnormal functioning of the prefrontal cortex associated with working memory deficits in patients with schizophrenia. The nature of these deficits, however, remains controversial. For example, fMRI studies have demonstrated both enhanced (Callicott, Bertolino et al. 2000; Manoach, Gollub et al. 2000), reduced (Callicott, Ramsey et al. 1998; Barch, Carter et al. 2001; Perlstein, Carter et al. 2001; Barch, Csernansky et al. 2002) or no difference (Honey, Bullmore et al. 2002; Walter, Wunderlich et al. 2003; Kindermann, Brown et al. 2004; Walter, Vasic et al. 2007) in DLPFC activation. Similarly, EEG studies examining gamma oscillatory activity in patients with schizophrenia while performing cognitive tasks have also produced inconsistent findings. For example, reduced induced oscillatory activity has also been reported in patients with schizophrenia while performing a cognitive control (Cho, Konecky et al. 2006; Lewis, Cho et al. 2008) and working memory (Lewis, Cho et al. 2008; Haenschel, Bittner et al. 2009) task. By contrast, increased evoked gamma oscillatory activity has been reported in patients with schizophrenia (Basar-Eroglu, Brand et al. 2007) and is consistent with the findings of studies one and three.

Divergent reduced and elevated gamma oscillatory activity findings may be accounted for performance differences in patients with schizophrenia compared to healthy subjects. That is, in fMRI, the parcelation of low versus high performing patients with schizophrenia during the N-back task has revealed differences in DLPFC activation and demonstrated reduced activity in low performers and enhanced activity in high performers (Callicott, Mattay et al. 2003). In addition, when high performing patients with schizophrenia are compared to high performing healthy subjects, increased activation of the DLPFC was revealed in patients (Callicott, Mattay et al. 2003). Consistent with the findings of Callicott et al (2003), study one also compared gamma
oscillatory activity at equivalent performance levels and found that patients with schizophrenia generated excessive gamma activity compared to healthy subjects. It is therefore possible that differences due to performance in patients with schizophrenia may account for the inconsistent fMRI and EEG findings. Furthermore, differences in EEG analytical methods may also contribute to these divergent findings at least during cognitive tasks. That is, the studies that employed induced EEG methods report reduced gamma activity (Cho, Konecky et al. 2006; Lewis, Cho et al. 2008; Haenschel, Bittner et al. 2009), while excessive activity is reported with evoked EEG methods (Basar-Eroglu, Brand et al. 2007; Barr, Farzan et al. 2010). As previously described, these methods differ in their phase relationship with the stimulus onset. That is, induced activity is not phase-locked to stimulus onset and appears as a jitter in latency that varies from trial to trial and therefore possesses a loose temporal relationship with the stimulus. Evoked oscillatory activity, by contrast, is phase-locked to the stimulus onset and is characterized by a constant time and phase relationship with the stimulus (Tallon-Baudry and Bertrand 1999).

However, induced EEG methods have been criticized for its susceptibility to both microsaccadic eye movement (Yuval-Greenberg, Tomer et al. 2008) and cranial musculature (Shackman 2010) artifact. It is therefore possible that the finding of reduced gamma activity through induced EEG methods may reflect microsaccadic or cranial musculature artifact that is canceled out when evoked EEG methods are employed (Barr and Daskalakis 2010). The nature of prefrontal abnormalities in relation to cognitive deficits in patients with schizophrenia continues to be debated. Moreover, factors such as performance levels, experimental paradigms and analytical methods, antipsychotic medication, and heterogeneity in DLPFC activation warrants further examination in assessing gamma oscillatory activity during cognition in patients with schizophrenia.
6.7 Specificity of rTMS on Frontal Evoked Gamma Oscillatory Activity in the DLPFC

In the series of studies presented in this PhD thesis we examined frontal evoked gamma oscillatory activity in the DLPFC. We examined the DLPFC because of its role in the mediation of working memory and the considerable evidence for this brain region in the pathophysiology of schizophrenia (Elvevag and Goldberg 2000; Deiber, Missonnier et al. 2007). In the series of studies presented here, rTMS was targeted at the DLPFC determined through a novel neuronavigational technique which mapped the coordinate position of the DLPFC based on meta-analyses on fMRI studies on working memory onto T1-weighted image of a MRI obtained for each subject (see Appendix A; (Rusjan, Barr et al. 2010). The application of rTMS over the DLPFC in studies two and three of this thesis resulted in the modulation of evoked oscillatory activity in the frontal region that was absent in posterior brain regions. However, a study that evaluated EEG coherence reported an increase in alpha power in both the frontal and the posterior parietal cortices following rTMS over the frontal region of healthy subjects (Jing and Takigawa 2000). Different brain regions have been shown to oscillate at their natural frequency range and the frontal cortex has been shown to naturally oscillate at higher frequencies such as beta and gamma compared to other brain regions (Rosanova, Casali et al. 2009). This study by Rosanova et al (2009) may therefore explain the specificity of rTMS on frontal evoked gamma oscillatory activity in healthy subjects. In patients with schizophrenia, though, study three showed that rTMS reduced frontal evoked delta oscillatory activity in the 3-back condition as well. As described earlier, interactions exist between the activities in the frequency bands due to their non-random relationships (Roopun, Kramer et al. 2008). That is, cross-frequency or “nesting” relationships describes when the power of a discrete frequency band is modified by the phase of a lower frequency band that coexists during information processing (Roopun, Kramer et
al. 2008). Such nesting interactions have been observed in the hippocampus (Lakatos, Shah et al. 2005) and neocortex (Penttonen and Buzsaki 2003) between delta, theta, and gamma frequency bands. Thus the finding of reduced frontal evoked delta and reduced gamma activity following rTMS in patient with schizophrenia may reflect the interaction between these two frequency bands.

6.8 Limitations and Future Directions

To our knowledge, the series of studies presented in this PhD thesis are the first to administer rTMS to measure its effects on frontal gamma oscillatory activity generated during a subsequent working memory task in patients with schizophrenia and healthy subjects. Although it was demonstrated that rTMS modulated this frontal evoked gamma oscillatory activity in both subject groups, these studies are not without their limitations. In this regard, the next section will discuss the collective limitations to this work as specific limitations have already been discussed within each study. In addition, future directions based on this body of work will also be explored.

First, studies two and three limited the examination of rTMS to the DLPFC, applied at 20 Hz frequency to both hemispheres on oscillatory activity during working memory. It is possible though that rTMS applied at a different frequency to the DLPFC may also result in the modulation of gamma activity that may also influence working memory performance. For example, Okamura et al demonstrated significant increases in the absolute gamma power following 10 Hz rTMS applied over the left prefrontal cortex (Okamura, Jing et al. 2001). It has also been reported that 10 Hz rTMS applied to the right DLPFC resulted in an increase in accuracy on a spatial working memory task in healthy subjects (Hamidi, Tononi et al. 2009). These studies therefore suggest that 10 Hz rTMS applied to the DLPFC results in both the potentiation of gamma power and an improvement in working memory performance. It should
also be noted that these two studies administered 10 Hz rTMS to different hemispheres. In this regard, determining the optimal rTMS site (i.e., right, left or bilateral) for the treatment of working memory deficits should be further investigated. Hamidi et al (2009) demonstrated right rTMS stimulation improved performance on a spatial working memory task which is consistent with the view that the right DLPFC is recruited for spatial tasks, while the left mediates verbal working memory (Smith and Jonides 1999). As such, it is possible that stimulation of the left DLPFC may improve performance on verbal working memory tasks such as the N-back used in the studies presented here. Future experiments examining optimal rTMS stimulation parameters in addition to the DLPFC site for different working memory tasks are greatly needed.

Studies two and three also limited rTMS administration to the DLPFC and did not examine its effect on oscillatory activity in other brain regions. The DLPFC was chosen as the optimal site in which to stimulate based on the considerable evidence for its involvement in the mediation of working memory, evidence for abnormal DLPFC activation during working memory tasks, and also for the implication of this brain region in the pathophysiology of schizophrenia. Nevertheless, neuroimaging studies have demonstrated that working memory function is not limited to the prefrontal cortex and recruits a network involving the prefrontal cortex and the posterior parietal cortex (Honey, Bullmore et al. 2002). It has further been suggested that working memory information is stored in more posterior parietal regions and this information is retrieved by the prefrontal cortex (Curtis and D'Esposito 2003; Postle 2006). These studies therefore suggest that rTMS applied to the posterior parietal cortex may also improve working memory function. In this regard, 10 Hz rTMS applied to the superior parietal lobule (Hamidi, Tononi et al. 2008) and 5 Hz rTMS applied to the parietal cortex (Luber, Kinnunen et al. 2007) has been shown to result in a significant reduction in reaction time in healthy subjects tested in a working memory task. Taken together, future studies that examine optimal sites for rTMS
stimulation in addition to the effect of different stimulation parameters are needed in the continual investigation of rTMS as a potential treatment option for working memory deficits in patients with schizophrenia.

Another limitation of studies two and three presented here relates to the evaluation of oscillatory activity following rTMS administration. Although it was demonstrated that rTMS modulated gamma oscillatory activity during a subsequent testing of the N-back administered within 20 minutes of the stimulation, it would be of interest to examine the effect of rTMS during working memory performance. In this regard, Hamidi et al (2010) recently examined the effect of 10 Hz stimulation applied to the postcentral gyrus and superior parietal lobule in healthy subjects while performing a working memory task (Hamidi, Slagter et al. 2010). Through independent component analysis, Hamidi argued that rTMS related artifact can be removed without influencing neurophysiological activity as electrical artifact is temporally and spatially predictable (Hamidi, Slagter et al. 2010). In this study, a quadratic relationship was demonstrated between potential peak amplitude and pulse number within the TMS train which was characterized by a decrease followed by an increase in amplitude. That is, rTMS was shown to result in both the depression and potentiation of neural excitability and thus the effects of rTMS are more complex than the simple linear summation of the neural response (Hamidi, Slagter et al. 2010). Hamidi et al are the first to deliver rTMS while recording EEG activity and demonstrate through independent component analysis, rTMS related artifacts can be removed. This study underscores the need to evaluate pulse to pulse changes induced by rTMS and whether these neurophysiological changes are related to stimulation frequency. Such examination of rTMS effects on-line will thus provide a greater understanding of the effects induced by rTMS on oscillatory activity in the subjects tested in studies two and three.
A final limitation of these studies relates to the paradigm used to assess working memory. We administered the N-back task which is a challenging working memory task however it does not allow for the examination of subprocesses of working memory. That is, despite the numerous theories that exist on working memory, it is accepted that this mechanism includes encoding, maintenance, manipulation, and retrieval subprocesses. In this regard, previous studies have shown alterations in oscillatory activity within these subprocesses (e.g., (Haenschel, Bittner et al. 2009; Ince, Pellizzer et al. 2009)). Future studies which evaluate the effect of rTMS on oscillatory activity specifically generated within working memory subprocesses may provide a better understanding of how to optimize rTMS as a potential tool in which to ameliorate working memory deficits in patients with schizophrenia.

6.9 Conclusion

The series of studies presented in this PhD thesis demonstrated that patients with schizophrenia generate excessive frontal evoked gamma oscillatory activity during the N-back task that may contribute to working memory impairment in this disorder. Furthermore, it was demonstrated that high frequency rTMS over the DLPFC inhibits excessive frontal evoked gamma oscillatory activity in patients with schizophrenia while potentiating this activity in healthy subjects when tested in a subsequent N-back test. Although these studies are not without their limitations, they provide a better understanding of the neurophysiological mechanisms that may contribute to working memory deficits in patients with schizophrenia and suggest that rTMS may be used as a potential cognitive enhancing tool in which to ameliorate these deficits possibly through repeated treatment sessions.
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Appendix A: Optimal TMS Coil Placement for Targeting the DLPFC Using Novel MRI-Guided Neuronavigation

7.1 Abstract

The dorsolateral prefrontal cortex (DLPFC) has been implicated in the pathophysiology of several psychiatric illnesses including major depressive disorder and schizophrenia. In this regard, the DLPFC has been targeted in many repetitive transcranial magnetic stimulation (rTMS) studies as a form of treatment to those patients who are resistant to medications. The ‘5-cm method’ and the ‘10-20 method’ for positioning the transcranial magnetic stimulation (TMS) coil over DLPFC have been scrutinized due to poor targeting accuracies attributed to inter-subject variability. We evaluated the accuracy of such methods to localize the DLPFC on the scalp in 15 healthy subjects and compared them to our novel neuronavigational method, which first estimates the DLPFC position in the cortex based on a standard template and then determines the most appropriate position on the scalp in which to place the TMS coil. Our neuronavigational method yielded a scalp position for the left DLPFC between electrodes F3 and F5 in standard space and was closest to electrode F5 in individual space. Further, we found that there was significantly less inter-subject variability using our neuronavigational method for localizing the DLPFC on the scalp compared to the ‘5-cm method’ and the ‘10-20 method’. Our findings also suggest that the ‘10-20 method’ is superior to the ‘5-cm method’ in reducing inter-subject variability and that electrode F5 should be the stimulation location of choice when MRI co-registration is not available.
7.2 Introduction

The abnormal functioning of the dorsolateral prefrontal cortex (DLPFC) has been implicated in the pathophysiology of several neuropsychiatric disorders including major depressive disorder (MDD) and schizophrenia. Moreover, such deficits in DLPFC functioning have been related to symptom severity and serves as a target for repetitive transcranial magnetic stimulation (rTMS) as an alternative treatment. Given the promising success of rTMS over the DLPFC in alleviating symptoms associated with MDD and schizophrenia, advancement in improving the localization of this brain region is invaluable to optimizing rTMS as a potential treatment.

Over the past decade the ‘5-cm method’ has been routinely used for targeting the DLPFC in transcranial magnetic stimulation (TMS) studies. With this method, the DLPFC is localized by stimulating the motor cortex and recording motor evoked potentials in the contralateral hand muscle (i.e., abductor pollicis brevis; APB), and then measuring 5 cm anterior from this position along a parasagittal line (George, Wassermann et al. 1995; Pascual-Leone, Rubio et al. 1996). Although the ‘5-cm method’ is easy to perform without the cost of expensive neuronavigational techniques, it has been criticized for its failure to take into account cortex morphology and often yield positions that fall too short (Herwig, Padberg et al. 2001). For example, an MRI-based neuronavigational study reported that the ‘5-cm method’ was found to correspond to Brodman’s area (BA) 6 (premotor cortex) or 8 (frontal eye field) in 15/22 subjects, while a position within BA 9 was found in the other 7 subjects. Such inter-individual variation for identifying the DLPFC through the ‘5-cm method’ is thought to be a result of both variations in the distance between the motor areas and the DLPFC, and also low inter-rater reliability in determining this distance. Most importantly, such variability may also account for inconsistent therapeutic results.
when using repetitive TMS (rTMS) over the DLPFC to treat patients with MDD. For example, a recent study examined DLPFC location variability of the ‘5-cm method’ with rTMS anti-depressive effects and found significantly reduced depression scores in individuals with DLPFC positions that were more lateral and anterior (Herbsman, Avery et al. 2009). Furthermore, our group showed that targeted rTMS to DLPFC functional coordinates guided by a meta-analysis on neuroimaging studies on working memory in MDD patients (Fitzgerald, Oxley et al. 2006) resulted in greater anti-depressive effects compared to the ‘5-cm method’ (Fitzgerald, Hoy et al. 2009). Together these studies demonstrate the pitfalls of the ‘5-cm method’ and provide clear support for the need for better localization techniques that are less susceptible to inter-subject and inter-rater variability to optimize rTMS as a treatment for MDD.

The international 10-20 system (Jasper 1958) that is typically used in electroencephalography (EEG) electrode placement has proven useful in linking external scalp locations to underlying cortex. To this end, previous studies have used the 10-20 system to target the left and right DLPFC by placing the TMS coil over the F3 and F4 electrode, respectively (Gerloff, Corwell et al. 1997; Rossi, Cappa et al. 2001). Herwig and colleagues (2003) provided further support in ascribing F3 and F4 from the 10-20 system to the underlying DLPFC cortical area (Herwig, Satrapi et al. 2003). In their study, 21 subjects were co-registered with an EEG cap and dynamic reference frame mounted on their head. With their neuronavigational technique based on frameless stereotaxy, a probe was placed over electrodes F3 and F4 and its position projected 15 mm down to the underlying cortex to provide X, Y, and Z coordinates in individual space. The authors then used a Talairach atlas to relate individual coordinates to Talairach coordinates (x, y, z) = -37, 27, 44 for the left DLPFC, which corresponded to the dorsal and superior edge of BA 9, bordering BA 8 in standardized space. The findings by Herwig et al (2003), therefore, were consistent with previous studies (Gerloff,
Corwell et al. 1997; Rossi, Cappa et al. 2001) that identified electrode F3 as the closest electrode to target the left DLPFC. Although using the F3 electrode (‘10-2 method’) to represent the left DLPFC may be an improvement from the conventional ‘5-cm method’, it is still limited in several ways. First, as the DLPFC is a functional area, a comparison between an individual T1-weighted MRI and the Talairach atlas may not adequately identify this area. Second, although the ‘10-20 method’ takes into account head size, it does not take into account head shape, an important consideration in studies using rTMS therapeutically. Third, this method also does not take into consideration the morphology of the underlying cortex so that simply placing TMS coil over F3 to target the left DLPFC is less than ideal. By contrast, localizing the DLPFC based on functional standardized coordinates (e.g. Talairach or MNI coordinates) and then determining its relative location on the scalp for TMS coil placement may enhance the likelihood that the desired cortical region is activated. This study, therefore, was designed to evaluate the accuracy of previous methods to localize the DLPFC compared to our novel neuronavigational method, which first estimates the cortical position based on a standard template and then determines the most appropriate position for the TMS coil on the scalp in healthy subjects.

7.3 Methods and Materials

Fifteen right-handed healthy volunteers were tested (mean age=35.1 years, SD=7.77 years, range=24–49 years; 10 men and 5 woman). Subjects gave their written informed consent and the protocol was approved (Centre for Addiction and Mental Health in accordance with the declaration of Helsinki). Exclusion criteria included psychiatric or medical illness, a history of drug or alcohol abuse.
7.3.1 Procedure

7.3.1.1 Magnetic Resonance Image (MRI)

A T-1 weighted MRI (Acquisition Type=3D, TR=8.892 ms, TE=1.792 ms, Inversion Time=300, Number of Averages=1, Slice Thickness=1.5, FOV=20 cm, Matrix=256x256) was acquired for all subjects with seven fiducial markers in place for future co-registration. The images were converted to isotropic voxels of side 0.86 mm and the position of the anterior commissure (AC) was identified. The images were then stored in 16 bits with values ranging from 0 to 3000. A threshold value of 250 defined the iso-surface representing the scalp.

7.3.1.2 Functional Coordinates for the left DLPFC

The results from recent meta-analyses on functional neuroimaging studies testing working memory in patients with depression and schizophrenia (Glahn, Ragland et al. 2005; Mendrek, Kiehl et al. 2005; Tan, Choo et al. 2005; Fitzgerald, Oxley et al. 2006) were used to identify the functional region of the left DLPFC. These studies, however, localized the left DLPFC to an area within the anterior portion of the medial prefrontal cortex at the juncture of BA 9/46, and did not specify a single coordinate position. Although a study conducted earlier by our group examined rTMS applied to DLPFC position using (x, y, z) = -45,45,35 in Talairach coordinates ((x, y, z) = -46, 45, 38 MNI coordinates) resulted in greater reduction in depressive scores in MDD patients, this does not preclude that other voxel positions within this juncture may more optimally improve the antidepressant effects of rTMS in MDD patients. As such, we selected the coordinate (x, y, z) = -50, 30, 36 after converting these coordinates to MNI space that also corresponded to the juncture of BAs 9 and 46. Our DLPFC coordinate (x, y, z) = -50, 30, 36 mm resulted in a position that was a little more posterior and lateral to those examined by Fitzgerald et al. (2009).
7.3.1.3 Optimal TMS Coil Placement for Targeting the Left DLPFC

Importantly, the neuronavigational method used to position the TMS coil on the scalp in this study, can be applied to any cortical coordinates in the brain. It involved two steps: we first found the position of the left DLPFC on the cortex of each subject (DLPFC\textsubscript{C}) based on the normalization of a template, followed by the application of a marching cubes algorithm to find the optimal position for the coil on the scalp (DLPFC\textsubscript{S}; Figure 1). These procedures were done for all 15 subjects included in this study.

7.3.1.3.1 Estimating the left DLPFC on the cortex (DLPFC\textsubscript{C})

Firstly the DLPFC (x, y, z=\(-50, 30, 36\); MNI brain) was identified on the brain template MNI/ICBM 152 (Evans 1993; Mazziotta, Toga et al. 2001) by meta-analyses on neuroimaging studies examining working memory in patients with MDD and schizophrenia (Glahn, Ragland et al. 2005; Mendrek, Kiehl et al. 2005; Tan, Choo et al. 2005; Fitzgerald, Oxley et al. 2006). These coordinates were also shown to result in superior therapeutic efficacy in MDD when targeted through rTMS compared to the ‘5-cm method’ (Fitzgerald, Maller et al. 2009). For each subject, the best non-linear transform to map the standard brain to each individual brain was estimated using the subroutine spm\_normalize from the SPM2 (www.fil.ion.ucl.ac.uk/spm/; Ashburner, Neelin et al. 1997; Ashburner and Friston 1999). Next, the coordinates for the DLPFC\textsubscript{C} in individual brains were estimated by applying the resulting transformation to the coordinates of the DLPFC\textsubscript{C} in the template (Figure 1; Step 1A).
Figure 2. A non-linear transformation to convert the coordinates from a standard space to an individual space was first estimated (Step 1A). This estimated transform was then applied to the coordinates for the DLPFC in the standard brain (or template) to determine the position of the DLPFC in the individual brain (Step 1B). Next, a triangular mesh wrapping the iso-surface representing the scalp was created with the marching cube algorithm (see Methods). The optimal position for the coil (mDLPFC\textsubscript{S}) on the scalp was then defined as the vertex of the triangular mesh with a normal that passed through the DLPFC on the cortex (DLPFC\textsubscript{C}; Step 2).
7.3.1.3.2 Estimating the position of the left DLPFC on the scalp (DLPFC_s)

The DLPFC_s on the scalp was defined as the position in which the tangential plane to the scalp had a perpendicular (called normal) that passed through the DLPFC_C. The position on the surface of the scalp was estimated using a classic algorithm for rendering called marching cubes (Lorensen 1987) followed by a selection process. To use the marching cubes algorithm, a threshold value of 250 (the value-tone color at the border of the scalp) was chosen to define an isosurface on the MRI representing the scalp. The marching cubes algorithm (http://local.wasp.uwa.edu.au/~pbourke/geometry/polygonise/) generates a triangular mesh (a set of 3-3D coordinates) rendering this 3D surface on the image. Note that in actuality this threshold value defines 3 surfaces in a T1 contrast: the exterior surface of the scalp, the interior surface of the skull and the surface of the cortex. In addition, to create the triangular mesh, this version of the marching cube algorithm determined a normal to the iso-surface at each vertex of the mesh averaging the normals to the faces of all the triangles that shared this vertex.

The selection process to determine the location of the DLPFC_C on the scalp consisted of 7 sequential steps:

1) The coordinates of each vertex was associated with the closest voxel and only the voxels with a normal that intersected a sphere of 3 mm around the DLPFC_C at a distance less than 3 cm were considered.

2) For each voxel (i) the average distance (d_i) to the other voxels were calculated. If \( d_i > 23 \text{mm} \), the voxel (i) was filtered out. This filtering process was necessary to remove voxels that were generated by the marching cubes algorithm that were further from the main cluster and were likely to be outliers.
3) A second filter was then used to remove voxels that were within 1.5 cm of the DLPFC, as these voxels were most likely to be on the cortex rather than the scalp.

4) Next, an average normal from all of the ensuing voxels (Figure 2A) was calculated, and those voxels with a normal that formed an angle greater than 25° with respect to the average were removed.

5) An average position and normal was then calculated from the remaining vertices.

6) A 3D image of a 6 cm line that represented a normal to the scalp (estimated in step 5) which also passed through the middle point of the vertices’ mean position was then superimposed on the MRI image. As quality control this line should have passed through the DLPFC.

7) The position of the coil on the scalp (DLPFC) was then determined by manually by selecting one point on this 3D line, which fell on the scalp. A spherical mark with a diameter of 2.5 mm (i.e., small than a fiducial mark) was then superimposed on the MRI image for later co-registration of the MRI image (Figure 2B). The position for the DLPFC predicted through our method will be called ‘method’ DLPFC (mDLPFC), while this position is referred to as the ‘experimental’ DLPFC (eDLPFC) once it is co-registered to the subjects’ MRI, representing its true position on the scalp.
Figure 2. A) In this coronal slice of a MRI image, the orange spot represents the DLPFC on the cortex (DLPFC\textsubscript{C}) and the green squares are voxels on the iso-surface with a normal that passes through the DLPFC\textsubscript{C} in a distance less than 3 cm. Voxels (green squares) on surfaces other than the scalp (i.e., the internal side of the skull, and the interface gray matter-CSF) were filtered out if they had a normal greater than 25° from the average normal. The \textit{m}\textsubscript{DLPFC\textsubscript{S}} was defined as the intersection of the scalp with a line with the orientation of the average normal and passes through their average position. B) The position of \textit{m}\textsubscript{DLPFC\textsubscript{S}} was superimposed on each individual's MRI for later co-registration. Once \textit{m}\textsubscript{DLPFC\textsubscript{S}} is co-registered, this position is referred to as the \textit{e}\textsubscript{DLPFC\textsubscript{S}}.
7.3.1.4 MRI co-registration

Prior to MRI co-registration, a 64 channel EEG cap (STIM2, Neuroscan, U.S.A.) was placed on the subjects’ head to mark the positions of electrodes AF3, F3, F5, FC3, and FC5 (‘10-20 method’) with an ink-filled syringe. The EEG cap was then removed for identification of the ‘5-cm method’.

In accordance to the ‘5-cm method’ to localize the DLPFC, single monophasic TMS pulses (Magstim Company Ltd., UK) were administered to the left APB of the motor cortex, while resulting electromyography activity was collected using commercially available software, Signal (Cambridge Electronics Design, UK). A felt pen was then used to mark the optimal position under the centre of the coil for eliciting motor evoked potentials from this muscle (APB) and the location of the DLPFC was measured 5 cm anterior from this position.

Neuronavigational techniques (MINIBIRD system; Ascension Technologies) combined with MRicro/reg software were first used to co-register the 7 fiducial markers to subjects’ MRI with the position of the mDLPFC\textsubscript{S} superimposed on the image (represented as a sphere in Figure 2B). The position of the DLPFC\textsubscript{S} was then marked on the subjects’ scalp and its experimental coordinates recorded, and referred to as eDLPFC\textsubscript{S}. Next, the positions marked on the scalp for APB, ‘5-cm method’, AF3, F3, F5, FC3, and FC5 electrodes (‘10-20 method’) were registered to the image and their coordinates recorded. These coordinate positions were first translated to the MNI/ICBM using an affine transformation from the individual brain to the template (i.e., the inverse of the affine transformation that was first used in the non-linear transformation to estimate DLPFC\textsubscript{C}). Finally, the positions APB, ‘5-cm method’, and AF3, F3, F5, FC3, FC5 electrodes (‘10-20 method’) relative to the eDLPFC\textsubscript{S} were also measured to provide data in individual space.
7.3.1.5 Software implementation

The algorithms were programmed in C++ and integrated in a friendly graphic user interface. SPM ran under MATLAB (The MathWorks, Inc. Natick, MA, USA), called from the C++ via the API interface. In total our method takes approximately 5 minutes with most of the time allotted to selecting the voxel location of the mDLPFC$_S$ (Step 7). The automated steps are practically instantaneous with any current hardware. Software is available by request via e-mail to pablo.rusjan@camhpet.ca.

7.3.2 Data Analysis

To evaluate the inter-subject variability found between all of the methods (i.e., ‘5-cm method’, ‘10-20 method’, and our method), a jackknife approach was used for each ellipsoid volume representing the dispersion (N=15) of each landmark:

1) The mean position and the standard deviation of each dimension ($\sigma_x^j, \sigma_y^j, \sigma_z^j$) for each method (condition) in each sub sample ($j$), by leaving one subject ($j$) out of the jackknife, where $j=1$-15 was calculated. The standard deviations were then used to estimate the dispersion as the volume of an ellipsoid:

$$\text{Vol}_{ellipsoid}^j = \frac{4}{3} \pi \sigma_x^j \sigma_y^j \sigma_z^j$$

2) The difference between the dispersion of methods within each sub sample was then calculated as the difference of ellipsoid volume.

3) Finally, Wilcoxon non-parametric signed-rank tests were used to determine whether the volumes derived under each method (i.e. the 3-dimensional amount of variability in estimates) differed. A non-parametric approach seemed more appropriate than a
series of paired t-tests, since the assumptions underlying parametric tests most likely would not have been met due to our sample size limitation. In addition, it may not have been appropriate to assume that the distributional properties of the composite standard deviation volume measurements were normally distributed.

7.4 RESULTS

7.4.1 Individual space

The positions of APB, the ‘5-cm method’, and electrodes AF3, F3, F5, FC3, and FC5 (‘10-20 method’) relative to the eDLPFCs were measured with a measuring tape and are shown in Table 1. These measures are not normalized by head size, and thus, provide an evaluation of both the ‘5-cm method’ and the ‘10-20 method’ to localize the left DLPFC in individual space. The average distance from APB to the eDLPFCs was approximately 5.3 cm, ranging from 3.0-7.3 cm in 15 subjects. Although 5.3 cm is close to 5 cm, this position was not on the same sagittal plane as the position given for the ‘5-cm method’, and the average distance between these two landmarks was 2.6 cm. In evaluating the ‘10-20 method’ to localize the left DLPFC, we found that F5 was the closest electrode to eDLPFCs with a mean difference of 1.9 cm, followed by electrodes FC5 (2.3 cm) and F3 (2.8), respectively (Table 1). Table 2, compares the voxel coordinates predicted by our method (i.e., the; Figure 2B) mDLPFCs with the position obtained with the co-registration probe during the experiment, referred to as eDLPFCs. Further, the difference between these two positions was 12.6 mm with the x-direction (left-right) driving this difference. More explicitly, the experimental coordinates were found to be more lateral (i.e., more exterior) than the centre of the fake fiducial marking the position of mDLPFCs (see Figure 3, same result in standardized space). This discrepancy may reflect some systematic bias of the method (i.e., manual selection of the voxel representing mDLPFCs) that could be related to the
intrinsic spatial resolution pattern of the MINIBIRD system, accuracy of the co-registration procedures or the need to take into account the physical characteristics of the scalp (i.e., amount of hair on the scalp, size of the probe). As such, the difference between these two locations (i.e., \( m_{DLPC_S} \) and \( e_{DLPC_S} \)) will be statistically tested in standardized space to account for differences arising from head size and MRI orientation.

7.4.2 Standardized space

In standardized space, the closest position to \( e_{DLPC_S} \) was found with the ‘5-cm method’ (Table 3). However, the average distance of \( e_{DLPC_S} \) to APB was approximately 7 cm and not along a parasagittal line as in the ‘5-cm method’. Figure 4 illustrates that DLPFC corresponds best to a position more lateral, inferior and posterior than F3, and superior to F5. In evaluating the inter-subject variability in ‘5-cm method’ and the ‘10-20 method’ compared to our method, Wilcoxon non-parametric signed-rank tests were performed (SAS System v.9.1.3; SAS Institute, NC, USA). Our method yielded the least amount of variation compared to the ‘5-cm method’ \((p<0.0001)\) and compared to the ‘10-20 method’ \((p<0.0001)\) for both electrodes F3 and F5, respectively. In addition, less inter-subject variability was found with ‘10-20 method’ compared to the ‘5-cm method’ \((p=0.0002, \text{ electrode F3}; p≈0.000, \text{ electrode F5, respectively})\). Finally, with all three methods, a greater standard deviation was observed in the anterior-posterior direction that may be reflective of the pattern of spatial resolution of the MINIBIRD, however, our method still yielded the least inter-subject variability for \( e_{DLPC_S} \) compared positions generated by the other methods.

Figure 3 compares the coordinates predicted by our method \( m_{DLPC_S} \) with the position obtained with the co-registration probe during the experiment, referred to as \( e_{DLPC_S} \) in standardized space. A square T-test (SAS System v.9.1.3; SAS Institute, NC, USA) was
performed between the $e\text{DLPFC}_S$ and $m\text{DLPFC}_S$ and found that the locations differed significantly (T-square=46.44, df=3.12, p=0.0004). Furthermore, a series of paired t-tests found that location of the $m\text{DLPFC}_S$ and $e\text{DLPFC}_S$ differed significantly in the x-dimension (p=0.0004), moderately in the y-dimension (p=0.0651), while there was no difference in the z-dimension (p=0.2947).
Figure 3. Although the position predicted by our method ($m$DLPFC$_S$; green) is placed on the subjects’ scalp, the actual position measured with the probe when co-registered ($e$DLPFC$_S$; orange) is biased in the radial direction. These positions are shown for all subjects in MNI space.
**Table 1:** Actual distance (cm) from the experimental location of left DLPFC ($e\text{DLPFC}_S$) to EEG electrodes, APB, and the ‘5-cm method’ on the scalp in all 15 healthy subjects in individual space.

<table>
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<th>F5</th>
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**Table 2:** A comparison showing the voxel coordinates predicted by our method ($m\text{DLPFC}_S$) with the voxel coordinates obtained during the experiment with the co-registration probe ($e\text{DLPFC}_S$) in individual space.

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**Table 3:** Positions in standard MNI space (mm) for each subject predicted by the method ($m$DLPFC$_S$) compared to positions measured during the experiment ($e$DLPFC$_S$).
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Figure 4. The dispersion of APB, ‘5-cm method’, eDLPFCs and electrode positions are represented by spheroids in standardized space. Spheroids are centered at the mean position for each measurement with a radius in each direction (i.e. X, Y, and Z) equal to 1 standard deviation. Please note that these positions were measured on the scalp so they are “flying” over the gray matter.
7.5 Discussion

Here we presented a neuronavigational method to determine the most appropriate position to place the TMS coil on the scalp in order to target left DLPFC, and compared this to the ‘5-cm method’ and ‘10-20 method’. We found that our neuronavigational method was the least susceptible to inter-subject variability followed by the ‘10-20 method’ and lastly the ‘5-cm method’ in standardized space. Furthermore, we found that our neuronavigational method yielded a DLPFC location more anterior and lateral than the ‘5-cm method’ and between electrodes F3 and F5 of the ‘10-20 method’. However, if MRI acquisition is not feasible, we recommend using the ‘10-20 method’ by positioning the TMS coil over the F5 electrode as we found that the ‘10-20 method’ was less susceptible to inter-subject variability compared to the ‘5-cm method’.

Consistent with Herwig and colleagues (Herwig, Padberg et al. 2001), inter-subject variability was found with the ‘5-cm method’, which was greater than the variability found with our method (eDLPFCs position) and the ‘10-20 method’ (Table 3; Figure 4). Also consistent with Herwig and colleagues (Herwig, Padberg et al. 2001), we found that the ‘5-cm method’ generated positions for the DLPFC that were posterior of our position for the DLPFC based on meta-analyses on neuroimaging DLPFC activation during working memory in patients with depression and schizophrenia (Glahn, Ragland et al. 2005; Mendrek, Kiehl et al. 2005; Tan, Choo et al. 2005; Fitzgerald, Oxley et al. 2006). Thus, the recommendations in this study are based on patients with depression and schizophrenia; however, the advantage of our neuronavigational method is that it can be employed to target any brain region.

The ‘10-20 method’ was found to be less susceptible to inter-subject variability compared to the ‘5-cm method’ and yielded positions closer to the DLPFC position obtained by our
method. Furthermore, when MRI coregistration is unavailable, the ‘10-20 method’ should be used to direct the TMS coil over the F5 electrode (Figure 4; individual space). This finding, therefore, suggests that the TMS coil should be centered on electrode F5 and by extension F6 (‘10-20 method’) to target the left and right DLPFC, respectively.

Our group has previously shown that targeting the DLPFC using neuronavigational techniques based using functional coordinates \((x, y, z) = -45, 45, 35\) (Talairach coordinates; \((x, y, z) = -46, 45, 38;\) MNI coordinates) for this area results in a significant reduction in scores on the Montgomery-Asberg Depression Rating Scale compared to those patients whose DLPFC was determined through the standard or ‘5-cm method’ (Fitzgerald, Hoy et al. 2009). The functional coordinates investigated in this study were guided by a previous meta-analysis on neuroimaging studies which examined working memory in patients with major depressive disorder (MDD; Fitzgerald, Oxley et al. 2006)). The results of the meta-analysis found the DLPFC to be located within the juncture of BAs 9 and 46. Although, using this coordinate position indeed resulted in greater treatment efficacy, it does not preclude that other voxel coordinates within this juncture may more optimally improve the anti-depressant effects of rTMS in MDD patients. In this regard, we selected our voxel coordinate for the DLPFC by first converting the coordinates used by Fitzgerald et al. (2009) to MNI coordinates that also were associated within the juncture of BAs 9 and 46. Our DLPFC coordinate resulted in a position that was a little more posterior and lateral to those examined by Fitzgerald et al. (2009). In line with our position for the DLPFC, a recent study (Herbsman, Avery et al. 2009) who examined the relationship between the variability of the DLPFC location determined by the 5 cm rule (i.e., ‘5-cm method’) with scores on the Hamilton Depression Rating Scale in MDD patients. Herbsman et al. observed greater clinical response to rTMS in individuals with DLPFC positions that were more lateral and anterior compared to individuals with more medial and posterior locations. The mean position of
the DLPFC was \((x, y, z) = -46, 25, 44\) (Talairach coordinates; \((x, y, z) = -47, 24, 48\); MNI coordinates) in those subset of subjects with more lateral and anterior positions. The DLPFC position examined in the current study \((x, y, z) = -50, 30, 36\); MNI coordinates) and the one in Herbsman et al. \((x, y, z) = -47, 24, 48\); MNI coordinates), thus, are more lateral and posterior to the coordinate previously investigated by our group (Fitzgerald, Hoy et al. 2009), and suggests that a more lateral position within the junction of BAs 9 and 46 may more optimally improve treatment efficacy in MDD.

The results of this study are limited in several important ways. First, our proposed method for determining the position of DLPFC\(_S\) assumes that the centre of the coil makes contact with the scalp in a single point therefore only one plane that was tangential to the scalp at this point were considered (Figure 3). However, hair and skin may have produced a different contact surface that may have caused some range of possible orientations for the TMS coil rather than a single tangential plane. This is consistent with the difference observed between the \(m\)DLPFC\(_S\) and \(e\)DLPFC\(_S\) locations in the \(x\)-dimension, which may have arisen from step 7 during the \(m\)DLPFC\(_S\) voxel selection, which is manually selected. In this step the scalp DLPFC location in the MRI was delineated as an arbitrary value of color intensity. A change in this threshold could move the scalp to a maximum in a voxel in either direction (1 voxel=0.86 mm). The true difference observed in the \(x\)-dimension between the two locations was equal to 10.4 mm in standardized space of which the size of the coregistration probe (8 mm), in addition to hair and skin may have contributed to this difference. The difference found between \(m\)DLPFC and \(e\)DLPFC positions, therefore, reflects a distance along the normal to the tangential plane that the TMS coil is positioned. Most importantly, regardless of where which voxel for the \(m\)DLPFC we select the along this line, the effect of the TMS on the DLPFC would not change and therefore is clinically insignificant. We have determined, however, that by selecting a voxel position that
lies above the scalp rather than one that intersects the scalp, the difference arising in the x-
dimension will be minimized. For example, in Figure 2B we can superimpose a line
representing the normal to the tangential plane on the scalp instead of a sphere. With this slight
modification of our voxel selection process, any differences between the mDLPFC and eDLPFC
positions would be owing to the variability in the MINIBIRD system itself. Second, since the
non-linear transformation (SPM) used in this study to convert the standard template to the
individual brain (Figure 1; Step 1) were designed to match the full brain rather than just the
cortex, other transforms that are based on the trajectory of deep sulci in the cortex (i.e., surface-
based techniques for warping three-dimensional images of the brain (Thompson and Toga 1996))
may increase the accuracy of the method proposed in this study. Future studies examining the
accuracy in using non-linear transformations for determining the position of Talairach
coordinates to individual cortex should be investigated. Furthermore, future studies should also
aim to improve the algorithms used for rendering the surface of the scalp. Although the
marching cubes algorithm (based on an arbitrary threshold value) seemed to work very well, this
tends to render other iso-surfaces (i.e., internal side of the skill and on the cortex) rather than just
the iso-surface of the subject’s scalp. Removing these extra iso-surfaces would decrease the
filtering processes and possible bias involved when the voxel for the mDLPFC s is selected and
therefore improve upon our method.

Although previous studies have also employed the marching cubes algorithm to
determine scalp positions to target other brain regions (Andoh, Riviere et al. 2009), this is the
first demonstration of neuronavigational methods which took into consideration the functional
coordinates of the brain region of interest and the orientation of the TMS coil in order to
optimally target the DLPFC. We used a novel neuronavigational method, which accurately
targeted the DLPFC with less inter-subject variability compared to both the ‘5-cm method’ and
‘10-20 method’. Our method improves upon these previous methods to localize the DLPFC in two respects. First, although neuroimaging studies report that the location of the DLPFC at the juncture of BAs 9 and 46, our group previously demonstrated that using functional coordinates more accurately determines the most appropriate position which to direct the TMS coil in order to optimize rTMS treatment efficacy in MDD (Fitzgerald, Hoy et al. 2009). We, therefore, account for the fact that this is a functional brain region that is not considered in either the ‘5-cm method’ or the ‘10-20 method’. Second, a marching cubes algorithm determined the optimal location for TMS coil placement on the skull from the cortical position, thereby accounting for cortex morphology, skull shape, and the orientation of the TMS coil. We, therefore, have minimized inter-subject variability inherent in these methods that do not consider characteristics of head and/or cortex or TMS coil orientation. Our neuronavigational method, therefore, demonstrates a significant advancement to accurately target the DLPFC in rTMS treatment studies.

The method proposed in this study is important for several reasons. First, previous studies have shown that targeted rTMS to the DLPFC results in greater clinical response in patients with MDD compared to non-targeting techniques (Fitzgerald, Hoy et al. 2009). Second, F5 from the ‘10-20’ system was found to be the closest electrode to target the DLPFC and may be used in clinical practice without an acquisition of a MRI. Finally, our method is straightforward, requires approximately 5 minutes to perform, and can be applied to any brain region.
Copyright Acknowledgements (if any)