Evaluating The Effects of Cannabis Dependence and Abstinence on Cortical Inhibition and Working Memory in Schizophrenia

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Sciences
University of Toronto

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**Background:** Cannabis is the most commonly used illicit substance among schizophrenia patients. GABA is inhibited by cannabis and dysfunctional GABAergic neurotransmission underlies cognitive and cortical inhibitory deficits in schizophrenia patients. This study investigated the relationship between cannabis dependence, schizophrenia, cortical inhibition and cognition.

**Methodology:** Using transcranial magnetic stimulation and the N-back task with electroencephalography, we assessed cortical inhibition and working memory at baseline and following 28-days of abstinence in 12 cannabis dependent patients versus 14 cannabis dependent non-psychiatric controls.

**Results:** Enhanced GABAB activity, through a prolonged cortical silent period, was found in patients compared to controls at baseline, with no differences following abstinence. No differences were found on working memory accuracy or gamma activity.

**Conclusions:** While these findings suggest that cannabis dependence may have more impairing effects on GABAB mediated inhibition in controls compared to patients, it is clear that continued research is needed to better understand this common co-morbidity.
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Contributions

The thesis was a subset of a larger study entitled “Effects of Cannabis Abstinence on Neurocognition in Schizophrenia”. This study was developed by Dr. Tony George (PI), Dr. Konstantine Zakzanis, Rachel Rabin, Dr. Jeff Daskalakis, and Dr. Mera Barr. The neurophysiologic subset of the study was funded by Dr. Mera Barr’s young investigator NARSAD award and by Dr. Tony George’s CIHR grant.

The MSc. candidate, Michelle S. Goodman, was responsible for recruitment, retention, and conduct of all study sessions. Additionally, Michelle conducted all data analysis, interpretation of research data, and drafting of the thesis, with assistance from Dr. Tony George and Dr. Mera Barr. Dr. Mera Barr trained the candidate on all neurophysiological and neurocognitive measures, including working memory and paired associative stimulation. Rachel Rabin was responsible for conducting the structured clinical interviews and physical examinations performed during the screening session.

The Program Advisory Committee (PAC) members, Dr. Daniel Blumberger and Dr. Martin Zack, assisted with the interpretation of the data and provided feedback on the thesis.
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1 millivolt (1 mV)
2-Arachidonoylglycerol (2-AG)
Abnormal Involuntary Movement Scale (AIMS)
Analysis of Variance (ANOVA)
Barnes Akathisia Rating Scale (BARS)
Biobehavioural Addictions and Concurrent Disorders Research Laboratory (BACDRL)
Carbon Monoxide (CO)
Cannabinoid receptor gene (CNR1)
Cannabinoid Type 1 Receptor (CB₁)
Cannabinoid Type 2 Receptor (CB₂)
Centre for Addiction and Mental Health (CAMH)
Chlorpromazine (CPZ)
Cigarettes per day (CPD)
Cortical silent period (CSP)
Diagnostic and Statistical Manual of Mental Disorders, 4th. Edition (DSM-IV)
Dorsolateral prefrontal cortex (DLPFC)
Electroencephalography (EEG)
Endocannabinoid (eCB)
Gamma-aminobutyric acid (GABA)
Grams per day (GPD)
Healthy Controls (HC)
Hopkins Verbal Learning Test (HVLT)
Intelligence Quotient (IQ)
Intercortical facilitation (ICF)
Megnetoencephaology (MEG)
Long interval cortical inhibition (LICI)
Positron emission tomography (PET)
Positive and Negative Syndrome Scale (PANSS)
Prefrontal cortex (PFC)
Research Ethics Board (REB)
Resting motor threshold (RMT)
Schizophrenia (SZ)
Short interval cortical inhibition (SICI)
Simpson Angus Rating Scale for Extrapyramidal Symptoms (SARS)
Standard Deviation (SD)
Statistical Package for the Social Sciences (SPSS)
Structured Clinical Interview for the DSM-IV (SCID-IV)
Transcranial Magnetic Stimulation (TMS)
Wechsler Test of Adult Reading (WTAR)
Working Memory (WM)
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Chapter 1
Literature Review

1.1 Schizophrenia

1.1.1 Phenomenology and Etiology

Schizophrenia is one of the most severe and debilitating brain disorders. While its prevalence is relatively low compared to other pervasive illnesses, at approximately 1%, schizophrenia remains to be one of the world’s leading causes of disability (Lewis & Lieberman, 2000; Whiteford et al., 2013). The early age of onset, high rates of unemployment, and frequent hospitalizations, highlight the immense personal and societal burden of this disorder (Rossler, Salize, van Os, & Riecher-Rossler, 2005).

The symptoms associated with schizophrenia are commonly characterized into three domains, including positive symptoms (e.g., delusions, hallucinations, thought disorganization), negative symptoms (e.g., blunted affect, alogia, anhedonia, social dysfunction, amotivation) and cognitive impairment (APA, 2000). Contrary to initial beliefs, current research supports the notion that functional outcome is better predicted by cognitive deficits rather than positive symptoms (Green, 2006). Importantly, these cognitive deficits appear prior to the onset of psychosis and generally persist following stable remission from positive symptoms (Bora, Yucel, & Pantelis, 2010). Unfortunately, these impairments along with negative symptoms are not sufficiently treated by current antipsychotic interventions.

The high degree of heterogeneity known to this disorder complicates both diagnostic and treatment approaches for those with schizophrenia (Andreasen, Arndt, Alliger, Miller, & Flaum, 1995; Davidson & McGlashan, 1997). This phenotypic heterogeneity is commonly observed in the age of onset and constellation of symptoms, response to treatment, as well as
the overall prognosis. Towards addressing this variability, diagnostic subtyping (paranoid, catatonic, disorganized, undifferentiated, and residual) was introduced in the DSM-IV TR; however, the efforts to validate these subtypes were deemed unsuccessful. In response, the DSM-V targeted clinical symptomatology as markers of the disease (American Psychiatric Association, 2013).

In addition to the complex presentation of this disorder, the etiology of schizophrenia is poorly understood. Current theories suggest that environmental and/or psychological stressors interact with specific genetic vulnerabilities contributing to the illnesses development. A range of environmental factors implicated in this disorder include, viral infections (Mednick, Machon, Hutunen, & Bonett, 1988), substance abuse (Moore et al., 2007) (refer to section 1.4.3) and urbanization (Krabbendam & van Os, 2005). Regarding the genetic predisposition, high heritability contributes to about 80% of the disorder’s liability (Gottesman, 1991). However, no one gene appears to be either sufficient or necessary for the development of schizophrenia (Tandon, Keshavan, & Nasrallah, 2008). Of note, neurobiological evidence suggests that these genetic alterations may be linked to abnormalities within neurotransmitter systems including dopamine, γ-aminobutyric acid (GABA) and glutamate (Harrison & Weinberger, 2005).

1.1.2 Pathophysiology of Schizophrenia

1.1.2.1 Dopamine

Dopamine is one of the most pervasive neurotransmitters in the central nervous system (CNS). The dopaminergic system has been implicated in numerous behavioural domains including information processing, memory, pleasure and addiction (Jones,
Importantly, aberrant dopaminergic functioning is thought to underlie the pathophysiology of several psychiatric disorders including schizophrenia. More specifically, the dopamine hypothesis of schizophrenia proposes that excessive dopaminergic transmission is involved in the onset of psychosis (Carlsson and Lindqvist 1963). This theory was initially developed resulting from the observation that dopamine-enhancing drugs, primarily amphetamines, induced psychosis-like symptoms in otherwise healthy individuals (Seeman and Lee, 1975). In support of this finding, later research revealed that the psychosis reducing properties of atypical antipsychotic medications were largely due to their D2 receptor blocking capabilities (Lieberman, Kane, and Alvir, 1987). While the first formulation of this hypothesis accounted for positive symptoms, it could not properly explain the negative symptoms and cognitive deficits inherent to this disorder. Subsequent neuroimaging, post-mortem and animal studies provided the basis for a reformulated hypothesis. This hypothesis incorporated hypodopaminergic activity in the prefrontal cortex (PFC), better accounting for such cognitive deficits and negative symptoms, along with the initial observation of hyperdopaminergic activity in other subcortical regions (reviewed in Davis, Kahn, Ko, & Davidson, 1991).

1.1.2.2 GABA

Dysfunctional GABAergic neurotransmission has also been implicated in the pathophysiology of schizophrenia (Tse, Piantadosi, & Floresco, 2014). As the primary inhibitory neurotransmitter in the brain, GABA supresses cortical activity by hyperpolarizing the cell and reducing the neuronal firing of several excitatory neurotransmitters including dopamine, noradrenaline, and serotonin. GABA is primarily synthesized from glutamate, and is comprised of two general classes of receptors: ionotropic GABA\(_A\) receptors and
metabotropic GABA_B receptors. GABA acts at inhibitory synapses in the brain, binding to both presynaptic receptors and postsynaptic receptors. This binding ultimately leads to hyperpolarization due to the influx of negatively charged chloride ions into the cell or positively charged potassium ions out of the cell.

Following the pivotal discovery of GABA’s role in inhibitory control, the GABA hypothesis of schizophrenia was developed (Roberts & Frankel, 1950). In support of this, post-mortem studies revealed numerous deviations in GABAergic functioning. For example, cortical reductions in the messenger RNA and protein for glutamic acid decarboxylase-67 (GAD 67), an enzyme necessary for the synthesis of GABA, have been found in the DLPFC (Akbarian & Huang, 2006) and other regions (Hashimoto et al., 2008) in individuals with schizophrenia. Furthermore, advances in neurophysiological methodologies have allowed researchers to investigate potential GABAergic neurotransmitter dysfunctions in vivo. Studies utilizing non-invasive brain stimulation techniques have provided insight into specific GABA_A and GABA_B receptor deficits in individuals with schizophrenia (Daskalakis, Christensen, Chen, et al., 2002; Farzan et al., 2010a; Takahashi et al., 2013). Similarly, position emission tomography (PET) studies have revealed that aberrant GABA functioning, specifically in the PFC and hippocampus, may underlie negative symptoms (Asai et al., 2008) and cognitive deficits in those with schizophrenia (Lewis, Volk, & Hashimoto, 2004).

Clinical findings and pharmacological studies have provided further support for the involvement of GABA in schizophrenia. For example, benzodiazepines have been shown to reduce the likelihood of experiencing a psychotic episode during relapse (Carpenter, Buchanan, Kirkpatrick, & Breier, 1999; Hashimoto et al., 2008). Given GABAs capacity to reduce synaptic dopamine levels, GABA was originally targeted as a potential treatment for
schizophrenia; however this approach proved to be somewhat unsuccessful. Instead of focusing on the GABA’s involvement in positive symptoms, researchers have directed their efforts towards better understanding the underlying association between aberrant GABA neurotransmission and cognitive dysfunction.

1.1.2.3 Glutamate

Over the past several decades, evidence supporting the involvement of glutamatergic neurotransmission in the pathophysiology of schizophrenia has grown substantially. Glutamate is not only a key precursor of GABA, it is also the most abundant excitatory neurotransmitter in the brain. Glutamatergic neurotransmission acts through metabotropic and ionotropic receptors, subdivided into N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isozolepropionic acid (AMPA) receptors, and kainite. Given its prevalence in the CNS, glutamate is thought to play a key role in several cognitive and behavioural domains including synaptic plasticity, learning and memory, and executive function (Johnson, 1972).

Early observations noted that phencyclidine (PCP) and ketamine, commonly abused NMDA antagonists, induced schizophrenia-like symptoms in otherwise healthy individuals (Javitt, 2007; Lahti, Holcomb, Gao, & Tamminga, 1999; Lahti et al., 1997) and significantly exacerbated positive symptoms in those with schizophrenia (Lahti, Weiler, Tamara Michaelidis, Parwani, & Tamminga, 2001). Thus, it was originally hypothesized that hypofunctional glutamatergic activity contributed to the development of schizophrenia. Post-mortem studies have provided support for this hypothesis, demonstrating decreased NMDA receptor densities in individuals with schizophrenia, specifically within the superior frontal (Sokolov, 1998) and temporal cortices (Humphries, Mortimer, Hirsch, & de Belleroche,
Furthermore, recent neuroimaging studies have revealed that along with psychotic-like symptoms, ketamine administration in healthy individuals led to abnormal cortical functional connectivity and activation (Driesen, McCarthy, Bhagwagar, Bloch, Calhoun, D'Souza, Gueorguieva, He, Ramachandran, et al., 2013; Driesen, McCarthy, Bhagwagar, Bloch, Calhoun, D'Souza, Gueorguieva, He, Leung, et al., 2013).

Taken together, this extensive clinical and preclinical research suggests that widespread neurotransmitter dysfunction underlies the pathophysiology of schizophrenia. These diverse set of findings highlight the fact that no one neurotransmitter alone can account for this complex brain disorder; as such, symptoms of schizophrenia likely arise from the dysfunctions within each of these neurotransmitter systems. It is clear however that many unknowns still exist regarding the pathophysiology and etiology of this disorder and continued research is necessary.

1.2 Cannabis

Cannabis, derived from the Cannabis sativa, Cannabis indica, or Cannabis ruderalis plant, has been used for centuries for its psychoactive and medicinal properties. Cannabis is most often used in the form of marijuana, a mixture of cut, dried, and ground flowers, leaves, and stems. Less common preparations include kief, a powder sifted from the leaves and flowers of the cannabis plant, and hashish, which is a concentrated resin formed from pressed kief. The primary psychoactive component in cannabis is delta-9-tetrahydrocannabinol (THC); however herbal cannabis contains over 400 additional chemical components and 60 cannabinoids including cannabidiol (CBD), cannabigerol and cannabinol (Iversen, 2008). These cannabinoid concentrations vary significantly among the different strains of marijuana. For example, cannabis sativa has been shown to contain high concentrations of THC, thus is
known for its psychoactive side effects, whereas cannabis indica is prepared from high CBD concentrations and thus is used for its sedative effects (Joy, 1999). Furthermore, cannabis potency (i.e., THC concentrations) varies substantially between its preparations. Cannabis or marijuana typically contains 5% THC, whereas resin and cannabis oils can contain THC concentrations as high as 20 and 60% respectively (United Nations Office on Drugs and Crime, 2009). In contrast, there is currently no consistent or reliable information concerning the concentrations of other cannabinoids including CBD, among the various cannabis strains.

Next to tobacco and alcohol, cannabis is the third most commonly used recreational drug (Iversen, 2003) and the single most commonly used illicit drug with approximately 150 million users worldwide (Cohen, Solowij, & Carr, 2008). It has been reported that 3.3 to 4.4% of the world’s population between the ages of 15-64 have used cannabis at least once in their lifetime (United Nations Office on Drugs and Crime, 2009). Among 20,000 individuals surveyed in North America, over 4% displayed signs of cannabis abuse and nearly 60% of these cases met criteria for cannabis dependence (Hall, Solowij, & Lemon, 1994). More specifically, in Canada, lifetime use has increased significantly from 23.2% in 1989 to 44.5% in 2004, with approximately 3% of Canadians reporting daily use (Adlaf, 2005).

1.2.1 Pharmacokinetics of Cannabis

The most common and most efficient method of cannabis administration is smoking. Cannabis is typically smoked as a joint or blunt, which differ in terms of the papers used to wrap the cannabis. Joints typically contain anywhere from 0.25 to 1 gram of cannabis and may be supplemented with tobacco, which aids in its burning. Additional methods of smoking include pipes or bongs as well as vaporizers. Vaporizers have become more popular
in recent years, as these devices are able to extract THC and other cannabinoids at temperatures lower than burning, thus eliminating the harmful secondary carcinogenic tars and carbon monoxide associated with smoking. Finally, cannabis may be consumed orally through “edibles”, which are typically used medicinally. Given that cannabis is hydrophobic, THC must be extracted using lipids or alcohol in order to preserve its psychoactive properties (Wolff & Johnston, 2014).

The pharmacokinetics of cannabis vary with respect to the route of administration. Regardless of the method of consumption, the high lipid-solubility of cannabis aids in its ability to quickly cross the blood-brain barrier. Cannabinoids accumulate in fatty tissue, yielding a long elimination half-life and persisting in the body for extended periods of time. THC is rapidly absorbed through the lungs following inhalation and is detectable in blood plasma within seconds. The psychotropic outcomes reach their maximum effect within 10 minutes, and can last up to 2-3 hours (Huestis, Henningfield, & Cone, 1992). The bioavailability varies in regards to depth of inhibition, breathhold, and puff duration. Additionally, THC may be lost in the butt of the joint, through sidestream smoke or through incomplete lung absorption (Grotenhermen, 2003). Furthermore, oral administration introduces more variability as well as delayed or diminished effects by up to 30%, due to the slower rate of absorption of THC from the digestive tract (Perez-Reyes et al., 1991).

THC metabolism takes place mainly in the liver, and to a lesser extent in the lungs and heart (Harvey, 1976). Initial hydroxylation results in a short-lived, potent psychoactive THC metabolite. Further oxidation in the liver converts 11-OH-THC to several inactive metabolites. To date, close to 100 metabolites have been discovered for THC (Harvey & Brown, 1991), including THC-COOH, the most abundant non-psychoactive metabolite in
plasma and urine (Adams & Martin, 1996; Hawksworth & McArdle, 2004). The exact elimination half-life of THC measured from plasma has proven to be difficult to calculate, due to variability in the dose, route of administration, period of observation and experience of the user. THC plasma elimination half-lives have been reported to range anywhere from 20 hours (Hunt & Jones, 1980) to up to 12 days (Johansson, Halldin, Agurell, Hollister, & Gillespie, 1989). A plausible explanation for this divergence may lie within the ability to distinguish THC from its other, longer lasting metabolites (Grotenhermen, 2003). Complete elimination has been shown to take as long as 12.9 days (ranging from 3-29 days) in light users to 31.5 days in chronic, heavy users (Ellis, Mann, Judson, Schramm, & Tashchian, 1985). Given the long elimination half-life, repeated cannabis use among chronic users leads to the accumulation of cannabinoids in the body and brain (Ashton, 1999), and has been suggested to lead to a form of “reverse tolerance” (Julien, 2001). However, there is no clear and consistent relationship between the plasma THC levels and behavioural effects (Agurell et al., 1986).

1.2.2 Acute Psychoactive Effects of Cannabis

The use of cannabis for its psychoactive and medicinal qualities dates back centuries (Zuardi, 2006). The psychoactive effects of cannabis are subjective and vary based on the experience of the individual, method of administration, dose and strain of cannabis. Cannabis use produces a variety of psychological and physiological effects; unlike other psychoactive substances, cannabis exhibits properties of hallucinogens, stimulants and depressants. Generally, mild cannabis intoxication acutely leads to euphoria, lethargy, perceptual and time distortions, and the overall augmentation of ordinary sensory experiences (Tart, 1970). At higher doses, side effects include short-term memory impairment, intense mood alterations,
and impaired motor coordination (Jaffe, 1985). While many individuals report anxiolytic effects following cannabis consumption, at higher doses, increased anxiety and panic reactions including paranoia and acute toxic psychosis are commonly reported (Ashton, 1999; Iversen, 2008).

1.2.3 Somatic and Health Effects of Cannabis

In addition to the psychoactive effects, cannabis produces a variety of acute somatic effects. While these side effects may only last for a short period of time, research has begun to uncover the long-term health outcomes associated with chronic cannabis use. For example, cannabis has been shown to lead to tachycardia, which has been suggested to accelerate the development of heart problems in at risk individuals. In support of this, studies have shown that the risk of myocardial infarction during the first hour after cannabis consumption increased 4.8-fold as compared to non-users (Hartung, Kauferstein, Ritz-Timme, & Daldrup, 2014; Mittleman, Lewis, Maclure, Sherwood, & Muller, 2001). Furthermore, research regarding the bronchopulmonary side effects of cannabis use have been reliably demonstrated. These effects include chronic inflammatory changes in the respiratory tract, which can manifest as chronic cough, wheezing, and phlegm (Van Hoozen & Cross, 1997). Additional adverse health effects include cancer, bronchitis and emphysema (Ashton, 1999). Finally, acute somatic effects of cannabis use include ocular redness, dry mouth and increased appetite commonly referred to as "the munchies" (Sansone & Sansone, 2014). It should be noted that the acute toxicity of cannabis is extremely low, as there have been no reported cases of human deaths directly from cannabis poisoning (Hall et al., 1994).
1.2.4 Cognitive Effects of Cannabis

There is a growing body of research investigating the impairing effects associated with cannabis use on cognition. Interestingly, comparisons have been drawn between impairments in perceptual, emotional, and cognitive domains accompanying cannabis use, and those seen in patients with schizophrenia (Radhakrishnan, Wilkinson, & D'Souza, 2014; Solowij & Michie, 2007). For example, studies have found that acute cannabis use impairs features of decision-making and planning including response times and accuracy (Ramaekers et al., 2006; Vadhan et al., 2007). Moreover, cannabis intoxication is associated with increased risk-taking through tasks assessing impulsivity and inhibition (McDonald, Schleifer, Richards, & de Wit, 2003). However, evidence regarding the impairing effects on attention and concentration is mixed, whereby the impairing effects seem to be stronger in less frequent users. In contrast, research suggests that attention in the chronic users may be disrupted more so by acute abstinence than intoxication (Crean, Crane, & Mason, 2011). These disparate findings may be due in part to the variability in study methodology, specifically associated with frequency and recency of cannabis use.

The long-term effects of cannabis use have received significant attention in recent years. Yet similarly to the findings regarding the acute effects, inconsistencies in the literature exist. Several studies have shown that cannabis appears to have continued impairing effects on executive function following a prolonged abstinence period. The most enduring deficits seem to lie within the domains of decision-making, concept formation, and planning (Verdejo-Garcia, Rivas-Perez, Lopez-Torrecillas, & Perez-Garcia, 2006). However, contrasting research has shown that cognitive deficits seen in long-term cannabis users may be eliminated following 28-day abstinence (Pope, Gruber, Hudson, Huestis, & Yurgelun-
The reversibility of these deficits indicates that chronic cannabis use may not permanently alter cognitive function in all domains.

1.2.5 Treatment of Cannabis Dependence

Treatment approaches targeting cannabis dependence have been adapted in a large part from alcohol use disorder interventions. However, compared to these better-established interventions, evidence-base research regarding the management of cannabis dependence is still somewhat lacking. In terms of behavioural interventions, motivational enhancement therapy (MET) and cognitive-behavioural therapy (CBT) have proven to be the most reliable and successful treatment approaches. MET focuses on resolving the users ambivalence about engaging in treatment and aims to enhance their motivation to change. In contrast, CBT addresses the skills necessary to quit or reduce ones substance use and change unhelpful thinking and behaviours that may impact treatment outcomes. Randomized control trials validating MET and CBT suggest that multicomponent therapy combining these treatments is most effective (Dennis et al., 2004; Stephens, Roffman, & Curtin, 2000).

A promising addition to these behavioural interventions is contingency management (Budney, Moore, Rocha, & Higgins, 2006; Kadden, Litt, Kabela-Cormier, & Petry, 2007). Current literature has provided support for the efficacy of contingency management in treating cannabis dependence in otherwise healthy individuals (Budney, Higgins, Radonovich, & Novy, 2000) and those with severe mental illness (Sigmon, Steingard, Badger, Anthony, & Higgins, 2000). Contingency management promotes behavioural changes through the use of positive or negative contingencies in an organized manner. In regards to substance use interventions, contingency management encourages behaviours
associated with treatment attendance and retention as well as decreasing drug use behaviours. Importantly, this method has proven to be effective in populations with low motivation for changing drug use behaviours (Sinha, Easton, Renee-Aubin, & Carroll, 2003).

Finally, there is a growing body of support for the use of pharmacotherapy targeting withdrawal symptoms associated with cannabis abstinence. However, this work is still in its early stages, as no government-approved medications currently exist. Furthermore, few studies have focused specifically on the physiological, subjective, and reinforcing effects of these medications. Preclinical evidence suggests that Δ⁹-THC, clonidine and lithium can effectively decrease withdrawal behaviours in rodents (Cui et al., 2001). In humans, nabiximols or Sativex, a cannabis extract with a 1:1 mixture of THC and CBD, was originally developed as a treatment for multiple sclerosis. However, interestingly, this medication has been shown to attenuate cannabis withdrawal symptoms. For example, in a treatment-seeking cohort, nabiximols reduced withdrawal symptoms and improved treatment retention. However, this medication did not effectively promote the long-term reductions in cannabis use following medication cessation (Allsop et al., 2014). These preliminary findings support the potential treatment efficacy of pharmacotherapy and highlight the need for continued research investigating the combined effects of behavioural and pharmacological interventions.

1.3 The Endogenous Cannabinoid System

The endocannabinoid (eCB) system is one of the most abundant neuromodulatory systems in the brain. This system is responsible for maintaining homeostasis within the CNS through its control over synaptic transmission (Pertwee, 2008). It is made up of two G-protein-coupled receptors, cannabinoid type 1 (CB₁) and cannabinoid type 2 (CB₂). The
discovery of the type 1 receptor led to the identification of natural occurring ligands or endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG). These endocannabinoids act at CB₁ receptors and are synthesized in response to increased intracellular calcium levels (Howlett et al 2002). Anandamide behaves similarly to THC, yet with less potent and pervasive actions (Iversen, 2008), acting as a partial agonist at both CB₁ and CB₂ receptors. In contrast, 2-AG is a complete agonist at both receptor subtypes. Cannabis, prepared in all of its forms, cannabis agonists, and endogenous ligands, act primarily on CB₁ receptors. While the two eCB system receptor subtypes are closely related, type 2 receptors are most often found on immune cells, and are involved in the immunological activity of leukocytes (Galiegue et al., 1995).

The complex interaction between CB₁ receptors and neurotransmitter systems are thought to mediate the principal effects of cannabis (Freund, Katona, & Piomelli, 2003). CB₁ receptors are primarily located on central and peripheral neurons (Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998) and are highly expressed throughout the brain. These brain regions, including, the hippocampus, cortex, cerebellum, basal ganglia, PFC, and anterior cingulate, are thought to be associated with cognition and mood regulation (Herkenham, 1991).

At the molecular level, CB₁ receptors are localized presynaptically on excitatory and inhibitory neurons, primarily on the axon terminals of GABA interneurons. These receptors are thought to mediate inhibition and thus maintain homeostasis by preventing excessive release of excitatory neurotransmission within the CNS (Eggan & Lewis, 2007). Evidence suggests that endocannabinoids act as retrograde signal messengers, whereby increases in postsynaptic intracellular calcium stimulated by specific neurotransmitters can trigger the
synthesis and release of endocannabinoids. These molecules are then thought to activate presynaptic CB₁ receptors and inhibit the further release of GABA (Pertwee, 2008; Szabo & Schlicker, 2005).

CB₁ activation through THC and other psychoactive cannabinoids enhances mesolimbic dopaminergic neurotransmission, which terminates at the PFC (Pistis et al., 2002), striatum (Bossong et al., 2009), and nucleus accumbens (Gardner, 2005). Converging clinical and preclinical research has implicated this increase in dopamine in both the cognitive impairments (Pistis et al., 2002; Pistis, Porcu, Melis, Diana, & Gessa, 2001) and reinforcing properties associated with cannabis use (Ameri, 1999; Bossong et al., 2009). The divergent effects of cannabis on GABA and dopamine may also underlie the subjective opposing stimulant and depressant effects experienced by individual users.

Similarly, the cannabinoids, specifically THC and CBD, have also been shown to possess divergent properties. THC acts as a partial CB₁ receptor agonist with relatively low receptor efficacy (Compton, Johnson, Melvin, & Martin, 1992; Pertwee, 1997). In contrast, CBD indirectly stimulates endocannabinoid signalling through suppressing fatty acid amide hydrolase (FAAH), an enzyme involved in the inactivation of endogenous cannabinoids (Piomelli, Beltramo, Giuffrida, & Stella, 1998). The molecular mechanism underlying CBD activity is not well understood, however it has been suggested that CBD has the ability to non-competitively antagonized CB₁ and CB₂ receptors (Murray, Morrison, Henquet, & Di Forti, 2007; Thomas et al., 2007). In this capacity, CBD has been shown to influence the pharmacological activity of THC, thus countering some of the undesirable side effects associated with THC administration (Englund et al., 2013; Zuardi, Crippa, Hallak, Moreira, & Guimaraes, 2006). This opposing molecule activity is reflected in the behavioural effects
of THC and CBD. For example, THC is known to induce paranoia in individuals with prior paranoid ideation (Freeman et al., 2015) as well as exacerbate pre-existing psychotic and affective symptoms in those diagnosed with schizophrenia (D'Souza et al., 2005). In contrast, CBD has been shown to possess antidepressant (Zanelati, Biojone, Moreira, Guimaraes, & Joca, 2010), anxiolytic (Bergamaschi et al., 2011; Zuardi, Shirakawa, Finkelfarb, & Karniol, 1982) and neuroprotective properties (Hayakawa et al., 2007).

1.4 Cannabis Use in Schizophrenia

Recent research has focused on cannabis use among individuals with schizophrenia due in part to its worldwide standing as the most commonly used illicit substance in this population. With approximately one-third of persons with schizophrenia and other psychoses reporting daily use (Jablensky, 2000) and one-quarter meeting criteria for a cannabis use disorders (CUD) (Koskinen, Lohonen, Koponen, Isohanni, & Miettunen, 2010), it is clear that understanding the molecular and behavioural effects of cannabis use in this population is essential.

1.4.1 Effects on Prognosis and Course

The presence of co-morbid cannabis use in schizophrenia is associated with devastating effects on prognosis and clinical severity. These individuals face symptom exacerbation, higher rates of relapse and poorer treatment compliance and success (D'Souza et al., 2005; Foti, Kotov, Guey, & Bromet, 2010; Linszen, Dingemans, & Lenior, 1994; Manrique-Garcia et al., 2014). Interestingly, studies that employ self-report measures generally find that patients use cannabis to relieve boredom, provide stimulation to feel good, to get high, or to relax and socialize with peers (Addington & Duchak, 1997; Fowler, Carr,
Carter, & Lewin, 1998; Goswami, Mattoo, Basu, & Singh, 2004). Of note, these self-report findings are susceptible to denial and rationalization. Yet these findings suggest that many patients are unaware of or may downplay the clear detrimental effects of cannabis use (McGee, Williams, Poulton, & Moffitt, 2000).

1.4.2 Effects on Cognition

Given the evident harmful effects of cannabis on the clinical course, it would follow that cannabis may have equally detrimental effects of cognitive functioning; however, current literature provides contradictory findings. A recent meta-analysis including 10 studies and 572 patients with schizophrenia with and without cannabis use, found that those with a history of cannabis use demonstrated better cognitive functioning compared to non-users. Furthermore, in the second study included in this meta-analysis, cannabis using first episode patients with schizophrenia performed better on several domains including visual memory, working memory and executive function when compared to first episode non-using patients (Yucel et al., 2012). Similar results were demonstrated in another meta-analysis focusing more specifically on the effects of cannabis by controlling for confounding poly substance use (Rabin, Zakzanis, & George, 2011). Researchers have suggested that these findings may be attributed to the neurochemical effects of cannabis (Coulston, Perdices, Henderson, & Gin, 2011), given that the neuronal pathways regulated by the eCB system are thought to underlie neurocognitive functioning (Gerdeman, Partridge, Lupica, & Lovinger, 2003). Alternatively, others have proposed that these cannabis-using patients may represent a subgroup of patients with better premorbid cognitive functioning and social skills (Dixon, Haas, Weiden, Sweeney, & Frances, 1991; Leeson, Harrison, Ron, Barnes, & Joyce, 2011; Rodriguez-Sanchez et al., 2010). As such, individuals in the subgroup are thought to possess
a potentially less severe form of the disorder, developing psychosis following the initiation of cannabis use (Yucel et al., 2012).

In contrast, several studies have demonstrated that cannabis use may further impair cognitive performance in patients with schizophrenia. This finding has been replicated in studies employing lab-based (D’Souza et al., 2005), cross-sectional (Ringen et al., 2010), and longitudinal approaches (Mata et al., 2008). Finally, several studies have been unable to show that this comorbidity has a significant or consequential effect on cognition (Jockers-Scherubl et al., 2007; Sevy et al., 2007). These divergent findings may be attributed in part, to a lack of control for confounding variables within studies as well as methodological discrepancies between studies.

1.4.3 Underlying Neurobiological Connection

The once popular hypothesis of ‘self-medication’ suggested the psychiatric illnesses put those at risk for later cannabis use as a means to ‘self-medicate’ and alleviate unpleasant symptoms of the disorder and medication side effects (Wittchen et al., 2007; Musty & Kaback, 1995). However, more recent evidence has shown that self-medication does not adequately account for the pattern of cannabis use among patients with schizophrenia (Kolliakou et al., 2015). As such, researchers have begun to investigate other potential explanations for this common co-morbidity.

In spite of its prevalence, many unknowns still exist regarding the neurophysiological impact of cannabis use among individuals with schizophrenia. Aberrant eCB system functioning has been implicated in the detrimental effects of cannabis use on the course of illness. Genetic variants of cannabinoid-related genes, including the cannabinoid receptor
gene (CNR1), represent likely candidates underlying the interaction between cannabis use and schizophrenia (Martinez-Gras et al., 2006). Furthermore, disturbances in the eCB system have been demonstrated through elevated anandamide levels in the cerebrospinal fluid in both prodromal and untreated patients with schizophrenia (De Marchi et al., 2003; Koethe et al., 2009). Additionally, increases in cannabinoid type 1 receptor (CB1R) densities have been observed in this population (Ceccarini et al., 2013; Dean, Sundram, Bradbury, Scarr, & Copolov, 2001).

The GABAergic system has also been implicated in this co-morbidity. Aberrant GABA functioning seen in patients with schizophrenia may be further exacerbated by the inhibitory influence of cannabis on this neurotransmitter. In fact, it has been suggested the deficits in GABAergic activity in patients, may increase the likelihood of engaging in cannabis use (Rabin, 2014). Evidence supporting this suggestion comes from the overlapping influence of GABA on cognitive function both in individuals with schizophrenia and cannabis users (Benes, 1998; Di Lazzaro, Ziemann, & Lemon, 2008; Hill, Froc, Fox, Gorzalka, & Christie, 2004).

1.5 Cortical Inhibition
1.5.1 Indexing Cortical Inhibition using Transcranial Magnetic Stimulation

Cortical inhibition refers to the neurophysiological process whereby GABA inhibitory interneurons selectively attenuate the activity of pyramidal neurons in the cortex. One technique used to evaluate cortical inhibition is transcranial magnetic stimulation (TMS). TMS was developed as a non-invasive technique used to assess the function of the corticospinal tract in vivo (Kobayashi & Pascual-Leone, 2003). TMS is based on the concept
of electromagnetic induction as outlined by Faraday’s Law, whereby an intense current pulses within the coil, producing a magnetic field orthogonal to the plane of the coil. This field passes unimpeded through the skull and in turn induces an electric field tangential to the skull and in an opposite direction to that of the current in the coil. The horizontal orientation of the current favorably activates horizontally-oriented interneurons. Thus, pyramidal neurons are activated transsynaptically (Rothwell & Rosenkranz, 2005).

Clinicians and researchers have used TMS for both therapeutic and diagnostic applications. In terms of its diagnostic ability, combining TMS with electromyography (EMG) allows for the exploration of cortical GABAergic function. Stimulation in the motor cortex by a single TMS pulse produces a corresponding contralateral motor evoked potential (MEP). The ultimate effect of the stimulation depends on several important factors, including the shape of the magnetic coil, the intensity of the current pulse driven through the coil and the interstimulus interval separating the various pulses. Commonly used stimulation parameters include the focal figure-of-eight coil with stimulation intensities corresponding to the 1 millivolt (mV), i.e. the intensity needed to produce a MEP of 1 mV peak to peak amplitude. These TMS paradigms have been shown to reliably assess the activity of the GABA\textsubscript{A} and GABA\textsubscript{B} receptor functioning in the motor cortex in human subjects (Chen, 2000; Fitzgerald et al, 2002a).

More specifically, paired pulse TMS (ppTMS) has been used to reliably assess both cortical inhibition and excitation and thus the function of several neurotransmitter systems. For example, cortical excitation can be indexed through the resting motor threshold (RMT) and intracortical facilitation (ICF). The RMT is defined as the minimal intensity that produces a MEP of >50 μV in 5 of 10 trials in the relaxed muscle of interest. In contrast, ICF
is measured by administering a subthreshold pulse prior to the onset of the test pulse by 7 to 20 milliseconds (Kujirai et al., 1993; Ziemann, Rothwell, & Ridding, 1996). There is strong evidence to suggest that cortical facilitation is mediated in part, by glutamatergic neurotransmission (Clark, Randall, & Collingridge, 1994).

Cortical inhibition is also assessed through paired pulse paradigms, differing primarily in interstimulus intervals. For example, short-interval intracortical inhibition (SICI), a measure of GABA_A activity, is measured by administering a subthreshold pulse proceeded by a suprathreshold test pulse within 1 to 5 milliseconds. GABA_B in contrast, can be measured through two different paradigms. The first is a ppTMS called long interval cortical inhibition (LICI), which utilizes two suprathreshold stimuli at interstimulus intervals ranging from 100 to 200 milliseconds. The second paradigm, cortical silent period (CSP), incorporates motor cortical stimulation superimposed on background EMG activity resulting in a cessation of EMG activity. Thus the duration of this silent period represents an index of GABA_B activity (Cantello, Gianelli, Civardi, & Mutani, 1992).

Evidence regarding the involvement of GABA_A and GABA_B underlying SICI and LICI, respectively, comes from the time course of effects associated with these neurotransmitters. Cortical stimulation has been shown to produce disynaptic slow and fast inhibitory postsynaptic potentials (IPSPs) (Davies, Davies, & Collingridge, 1990). These fast IPSPs are thought to be mediated by GABA_A activity as they last approximately 20 ms, whereas slow IPSPs, mediated by GABA_B receptors, peak around 150–200 ms. This time course is reflected in the interstimulus intervals associated with SICI (1-5 ms) and LICI (100-200)(Sanger, Garg, & Chen, 2001). Additional support from pharmacological studies has revealed that administration of GABA_A receptor agonists, specifically lorazepam, reliably
enhance SICI whereas baclofen, a GABA\textsubscript{\text{B}} receptor agonist prolongs CSP (Di Lazzaro et al., 2008).

1.5.2 Cortical Inhibitory Deficits in Schizophrenia

While TMS has provided a reliable index of cortical inhibition and excitation in the motor cortex in healthy controls (Chen, 2000) its more important use has been to better understand changes in cortical functioning in individuals with mental illness (Daskalakis, Christensen, Chen, et al., 2002; Di Lazzaro et al., 2004; Maeda, Keenan, & Pascual-Leone, 2000). Aberrant cortical inhibition has been implicated in the pathophysiology of schizophrenia (Lewis, Pierri, Volk, Melchitzky, & Woo, 1999). Many early studies investigating the physiological impact of schizophrenia in the motor cortex have reliably demonstrated dysfunctional inhibition, yet their initial understanding of the mechanisms associated with these deficits was limited (Davey, Puri, Lewis, Lewis, & Ellaway, 1997; Puri, Davey, Ellaway, & Lewis, 1996).

Advances in our understanding of the specific neurotransmitters indexed through each TMS paradigm has allowed for a better understanding of the neurotransmitter deficits underlying schizophrenia as well as the impact of antipsychotic medication on these deficits. For example, Daskalakis and colleagues compared the RMT, CSP, SICI and ICF in 15 unmedicated patients with schizophrenia, 15 medicated patients with schizophrenia, and 15 healthy controls. The researchers found that the unmedicated patients exhibited the most profound cortical deficits on measures of CSP and SICI. Additionally, fewer deficits were observed among the medicated group when compared to the healthy controls (Daskalakis, Christensen, Chen, et al., 2002). These findings have been replicated in first episode patients as well as those with chronic schizophrenia (Fitzgerald, Brown, Daskalakis, & Kulkarni,
2002a; Fitzgerald et al., 2003; Wobrock et al., 2009). One of the more reliable and interesting findings regarding CSP comes from studies investigating the effects of antipsychotic medication on GABA\textsubscript{B}-mediated inhibition (Kaster et al., 2015). One such study found a significant lengthening of the CSP in patients treated with clozapine when compared to both unmedicated patients and controls (Daskalakis, Christensen, et al., 2008). Of note, a similar study demonstrated that this CSP finding was clozapine specific, as patients treated with any other antipsychotic medications failed to demonstrate this lengthened silent period (Liu, Fitzgerald, Daigle, Chen, & Daskalakis, 2009). These results have significant implications for the treatment of schizophrenia and highlight the importance of underlying GABA\textsubscript{A} and GABA\textsubscript{B} abnormalities in the pathophysiology of this disorder.

Reduced SICI among individuals with schizophrenia remains the most consistently replicated finding. These deficits have been demonstrated in patients with varying levels of illness severity and duration, including those at risk for developing this disorder (Hasan et al., 2012), first episode patients (Wobrock et al., 2008; Wobrock et al., 2009) and individuals with chronic schizophrenia (Daskalakis, Christensen, Chen, et al., 2002; Fitzgerald, Brown, et al., 2002a; Pascual-Leone, Manoach, Birnbaum, & Goff, 2002). In a recent meta-analysis conducted by Radhu and colleagues (2013), researchers found significant deficits in SICI in patients with schizophrenia, after controlling for age and medication, using a meta-regression. Furthermore, this finding showed specificity as a characteristic of schizophrenia, when compared to both patients with major depression and those with obsessive-compulsive disorder (Radhu et al., 2013).

However, some studies have been unable to find significant diagnostic differences in SICI (Daskalakis, Christensen, et al., 2008; Eichhammer et al., 2004; Liu et al., 2009) and
ICF (Eichhammer et al., 2004; Hasan et al., 2012; Wobrock et al., 2008) when comparing patients to controls. Finally, there are inconsistencies in the current literature regarding GABA_{B}-mediated inhibitory control in patients. For example, studies have revealed decreased (Daskalakis, Christensen, Chen, et al., 2002; Fitzgerald, Brown, Daskalakis, deCastella, & Kulkarni, 2002) increased (Bajbouj et al., 2004; Soubasi et al., 2010) and no differences (Davey et al., 1997; Frantseva et al., 2008; Puri et al., 1996) on measures of LICI and CSP.

Importantly, there is strong evidence to suggest that these cortical inhibitory deficits are associated with symptoms of schizophrenia. Liu and colleagues found that CSP duration was significantly inversely associated to negative symptoms, while positive symptoms were correlated with SICI. This finding was most pronounced in the unmedicated group (Liu et al., 2009). Thus these findings highlight the differential role that GABA_{A} and GABA_{B} likely have on the symptoms of schizophrenia. In a related study, researchers investigated the underlying association between abnormal cortical inhibition and social cognitive deficits. SICI and LICI and several dimensions of social cognition including, social perception and emotion processing, were evaluated in 33 unmedicated patients, 21 medicated patients and 45 controls. Inline with previous studies, SICI deficits were observed in the unmedicated group indicating less inhibitory response. Additionally, among the unmedicated group, deficits in SICI were associated with poorer scores on the measures of global social cognition and emotion processing, thus demonstrating the potential contribution of aberrant cortical inhibition to deficits of executive function (Mehta, Thirthalli, Basavaraju, & Gangadhar, 2014).
A major limitation to the above-mentioned TMS findings is that such measures were recorded in the motor cortex. Recent technological advances have allowed for the evaluation of cortical inhibition directly from the DLPFC through combined TMS with EEG (Daskalakis, Farzan, et al., 2008; Fitzgerald et al., 2008). Assessing cortical inhibition in the DLPFC is of particular importance in the field of mental illness research, as this region is associated with the pathophysiology, and cognitive deficits underlying SCZ and is the region where CBI receptors are densely populated.

Although this method is fairly novel, its validity and reliability have been well established in healthy subjects by comparing cortical evoked activity as measured through TMS-EEG with EMG activity from motor cortical inhibition (Farzan et al., 2010b). Using a pharmaco-TMS-EEG approach, researchers have been able to study the direct role of GABAergic neurotransmission through TMS-evoked EEG potentials (Premoli et al., 2014). Furthermore, this technique allows for the decomposition of the EEG signal into five well-established frequency bands to evaluate their inhibitory activity. Using this technique in patients with schizophrenia, previous research has demonstrated that DLPFC gamma inhibitory activity (30-50 Hz) is selectively impaired in individuals with schizophrenia compared to patients with bipolar disorder (Farzan et al., 2010b), obsessive-compulsive disorder and healthy individuals (Radhu et al 2015). This finding, along with additional evidence of decreased cortical inhibition in schizophrenia, provides further support for the role of the possibility that altered cortical connectivity may underlie the pathophysiology of schizophrenia (Frantseva et al., 2014).
1.5.3 Cortical Inhibitory Deficits with Cannabis Use

There is currently a paucity of research investigating the functional effects of cannabis use on cortical inhibition. Only one study has sought to determine whether individuals with chronic cannabis use demonstrate abnormalities in cortical inhibition. Fitzgerald et al (2009) examined the level of use (heavy versus light users) in forty-two cannabis users compared to 19 non-using controls on measures of cortical inhibition and excitation. Heavy cannabis use was defined as those who used 7 or more times a week and had consumed within 48 hours prior to testing. In contrast, light users were those who had used between 1 to 4 times a week, and had used sometime within the last 7 days of testing (Block & Ghoneim, 1993). TMS paradigms included SICI, ICF, LICI and CSP and THC plasma levels were measured prior to the TMS procedure. The researchers found that both heavy and light cannabis users demonstrated reductions in SICI compared to healthy controls, with no other differences on the additional measures of inhibition or excitation. Given that both heavy and light users exhibited comparable SICI deficits, the researchers suggest that this finding is likely dependent on long term neurobiological changes associated with lifetime cannabis use rather than the direct effect of cannabis on GABA\(_A\) activity. Thus, long-term cannabis use likely results in the down regulation of cortical inhibitory activity (Fitzgerald, Williams, & Daskalakis, 2009).

1.5.4 The Combined Effects of Cannabis and Schizophrenia on Cortical Inhibition

To date, only one study has investigated the effects of cannabis use on cortical inhibition in patients with schizophrenia. Twelve first-episode patients with a history of co-morbid cannabis abuse and 17 first-episode patients with no history of cannabis use
completed several TMS motor cortical paradigms including RMT, SICI, ICF, and CSP. Researchers found that stimulation to the right motor cortex in the cannabis-using group evoked lower cortical inhibition through SICI and enhanced facilitation as measured by ICF. In line with Fitzgerald et al. (2009), no group differences were observed in RMT or CSP duration. Interestingly, there were no significant correlations between psychopathology, disease characteristics, or the extent of cannabis abuse, with TMS parameters. Researchers suggested that their results likely reflect the ability of cannabis to exacerbate reduced cortical inhibition and enhanced facilitation in patients with schizophrenia (Wobrock et al., 2010). It is clear that continued research is needed to verify these results and determine if these same findings can be replicated in individuals with varying illness severity and duration.

1.6 Working Memory

1.6.1 The Neurophysiology of Working Memory

A key component of cognition that has garnered significant attention is working memory. Working memory is commonly defined by the ability to maintain and manipulate information for short periods of time (Baddeley & Hitch, 1974b; Barak & Tsodyks, 2014). As a higher order cognitive function, working memory is thought to be central to, and interact with several additional cognitive domains including learning, comprehension and reasoning (D’Esposito, 2007). As a theoretical construct, working memory has been defined through multiple models, based primarily on the discipline it is associated with, including cognitive psychology and cognitive neuroscience (Baddeley, 2003). One of the most significant contributions to the field was put forth by Baddeley and Hitch, who in 1974, described a model that combined the ability to both mediate executive control and maintain temporarily stored information (Baddeley & Hitch, 1974a).
Current working memory models came about following early preclinical observations, which guided their understanding of the brain regions associated with this cognitive domain. In 1936, Carlyle Jacobsen proposed that the PFC was integral to working memory functioning, following the observation that damage to this area in primates led to significant memory deficits. Interestingly, he found that the monkeys could complete complex cognitive tasks as long as the stimulus of interest remained present. However, the introduction of a delay period or the removal of the stimulus significantly decreased performance to chance (Jacobsen, 1936). Later work conducted by Joaquin Fuster supported this initial observation, whereby prefrontal neurons in monkeys were found to fire most rapidly during the delay period (Fuster, 1973). These early findings posed as the foundation for later working memory models, which suggest that the PFC contributes to working memory by filtering out extraneous information as well as maintaining and organizing key information in order to aid in goal directed behaviours (Cohen et al., 1997).

Methodological advancements have revealed that working memory function is not based solely on the PFC, but rather a complex network spanning frontal and parietal areas (Honey et al., 2002). However, there still exists debate regarding the role of each brain region. It has been suggested that the PFC may be involved in attentional activation, selecting strategies, and the manipulation of memory, where as the posterior areas are thought to play a role in the maintenance of information (Curtis & D'Esposito, 2003; Postle, 2006). However, neuroimaging and position emission tomography (PET) studies have provided a different method of categorization suggesting that the PFC may be associated with more specific types of memory, for example verbal working memory (Jonides et al., 1998; Smith, Jonides, Marshuetz, & Koepppe, 1998). Such lack of consensus regarding the functional brain regions
associated with working memory may be attributed to the type of working memory assessed or task difficulty.

Further complicating our understanding of the physiology of working memory lies within the complex coordination between excitatory and inhibitory neurotransmitter circuits. Goldman-Rakic and others have shown that neuronal prefrontal circuits aid in informational maintenance through recurrent excitatory glutamate outputs that fire throughout the delay period (Goldman-Rakic, 1995). These circuits are thought to be “tuned” by inhibitory GABAergic interneurons (Rao, Williams, & Goldman-Rakic, 2000). Thus GABA is believed to synchronize neuronal activity during working memory processes (Lewis, Hashimoto, & Volk, 2005). In support of this, Sawaguchi et al (1989) injected bicuculline, a GABA antagonist, into the DLPFC of monkeys, which lead to disrupted working memory performance (Sawaguchi, Matsumura, & Kubota, 1989). However, this finding is not specific to GABA; rather, research suggests that the coordination of several key neurotransmitter systems are necessary for optimal working memory performance (Robbins & Arnsten, 2009).

1.6.2 Indexing Working Memory

1.6.2.1 Neurocognitive Assessments

There are numerous well-established neuropsychological batteries used to assess working memory, which allow for the assessment of several memory domains both verbal and non-verbal (e.g., visuospatial, and audiospatial). Common non-verbal tasks include the delayed match to sample task and the spatial delay response. These non-verbal tasks are generally comprised of a brief presentation of a stimulus followed by a delay period. The participant is then required to either determine the original position of the stimulus (i.e.,
spatial delay response) or determine if an additional stimulus presented is the same as the initial one (i.e. delayed match to sample). In contrast, common verbal tasks include the number-letter-span, the Sternberg task and the N-back task. These tasks require participants to either maintain and manipulate a string of numbers or letters, through repetition and reordering (i.e., number-letter-span) or determine if the current letter/number presented was included in the original string (i.e. the Sternberg task). Finally, in the N-back task, letters are presented sequentially and participants are asked to determine if the same letter was presented N (i.e. 0-3) trials back, where 0 represents a control condition measuring reaction time only. Importantly, the N-back requires continuous updating with each trial, differentiating itself from other working memory tasks. Furthermore, the N-back task is commonly used in neurophysiologic-based studies given that it requires minimal movement for responses, thus decreasing the amount of muscle movement noise in the data.

1.6.2.2 Neurophysiological Assessments

Advances in brain imaging and recording techniques have provided novel insight into the neurophysiological underpinnings associated with working memory. Electroencephalography (EEG) is a commonly used technique, due to its high temporal resolution, ease of use, and low costs. This technique records brain activity from electrodes placed on the scalp, thus measuring a summation of synaptic activity from thousand of neurons in a similar spatial orientation from the outer layers of the brain. EEG is able to capture oscillatory activity in five discrete frequency bands including delta (1-4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (12-30 Hz), and gamma (30-50 Hz). Each oscillatory frequency is associated with specific brain states. For example, higher frequencies have been shown to be associated with the awake brain including cognitive tasks such as working memory, while
lower oscillatory activities are associated with the different stages of sleep (Buzsaki, 2006; Buzsaki & Draguhn, 2004). Synchrony among large neuronal networks is thought to be a critical component of healthy cognitive function (Engel, Fries, & Singer, 2001; Uhlhaas & Singer, 2006).

Gamma activity specifically, has garnered significant attention due to its involvement in working memory processes, as demonstrated in vivo through EEG and MEG studies (Mainy et al., 2007; Tallon-Baudry, Bertrand, Peronnet, & Pernier, 1998). Increases in gamma power are associated with increasing working memory load, most noticeably in the frontal brain region encompassing the DLPFC (Howard et al., 2003; Meltzer et al., 2008; Pesaran, Pezaris, Sahani, Mitra, & Andersen, 2002). Evidence suggests that GABA<sub>A</sub> receptor mediated IPSPs influence the generation of gamma oscillations (Bartos, Vida, & Jonas, 2007; Wang & Buzsaki, 1996; Whittington, Traub, & Jefferys, 1995), whereas GABA<sub>B</sub> is involved in the modulation of gamma oscillations (Brown, Davies, & Randall, 2007; Leung & Shen, 2007).

### 1.6.3 Working Memory Deficits in Schizophrenia

Working memory deficits among individuals with schizophrenia are one of the most replicable and robust findings in this population. A meta-analysis was conducted across 187 studies in patients with schizophrenia on three heterogeneous domains of working memory including verbal working memory, visuospatial memory and executive working memory. Researchers found clear deficits across all memory domains in the patient group compared to controls. Of note, no relationship was found between IQ and working memory performance, thus confirming that these deficits are not simply a function of low IQ (Forbes, Carrick, McIntosh, & Lawrie, 2009).
Neurophysiological research has suggested that in individuals with schizophrenia, working memory deficits may be attributed to dysfunction of the DLPFC, aberrant brain network connectivity, as well as disruptions in neurotransmitter signalling and neuronal synchrony (Cho, Konecky, & Carter, 2006; Lewis, Curley, Glausier, & Volk, 2012; Lewis & Moghaddam, 2006; Sun et al., 2011; Uhlhaas & Singer, 2010). However, inconsistent findings exist in the literature. For example, a meta-analysis including 12 neuroimaging studies in patients with schizophrenia found significantly lower DLPFC activation in patients during the N-back task (Glahn et al., 2005). However, a less conclusive meta-analysis including 29 studies failed to find a significant difference in frontal activation in patients compared to controls during a memory task (Van Snellenberg, Torres, & Thornton, 2006).

Additionally, several studies have demonstrated abnormal neural oscillatory activity in individuals with schizophrenia, predominantly in gamma frequency band (Farzan et al., 2010a; Gandal et al., 2010; Uhlhaas et al., 2006). EEG studies in epileptic patients (Howard et al., 2003) and healthy subjects (Barr et al., 2009; Cho et al., 2006) have reported increases in the modulation of gamma oscillations in the DLPFC with increased working memory load. In contrast, patients with schizophrenia seem to exhibit little to no gamma modulation in response to changing cognitive demands (Barr et al., 2010; Cho et al., 2006). It has been suggested that patients therefore inefficiently enlist complex cognitive processes to carry out tasks with low cognitive demands whereas healthy controls vary cognitive strategies in response to task difficulty (Basar-Eroglu et al., 2007). However, inconsistencies in the literature exist regarding gamma oscillatory activity evoked during working memory tasks in patients (Basar-Eroglu et al., 2007; Cho et al., 2006; Haenschel et al., 2009; Lewis et al., 2008). For example, Barr et al., (2010) demonstrated that compared to healthy controls,
patients with schizophrenia elicited excessive frontal gamma power, specifically during the 3-back condition of the N-back task. Such excessive gamma power was observed at equivalent performance levels between patients and healthy controls. Interestingly, the authors also found that task performance was inversely correlated to negative symptoms but not with positive symptoms (Barr et al., 2010). In contrast, a recent study conducted by Chen et al., (2014), found that deficits in gamma band amplitude in patients with schizophrenia during a modified Sternberg working memory task was associated with poorer performance. Moreover, working memory-induced gamma oscillations were dependent on baseline GABA levels across all participants (Chen et al., 2014). These findings provide further support for the importance of GABA function in the production of working memory specific gamma oscillations, even in those with aberrant system and cognitive functioning. While discrepancies in the research exist, taken together these findings suggest that neurobiological alterations in PFC activation, neurotransmitters and neural oscillations contribute to working memory deficits in schizophrenia.

1.6.4 Cannabis’ Effects on Working Memory

Only recently have researchers begun to accurately assess the effects of cannabis on working memory, better understanding and controlling for important confounding factors including frequency of previous cannabis use (Gruber, Sagar, Dahlgren, Racine, & Lukas, 2012; Solowij et al., 2011), and strain of cannabis consumed (i.e., the ratio of THC:CBD) (Morgan, Schafer, Freeman, & Curran, 2010). These well controlled studies in conjunction with advances in neuroimaging methodologies have provided a more conclusive understanding of the acute and long-term effects of cannabis as well as the potential mechanisms underlying this relationship.
There is an abundance of research demonstrating that acute administration of THC has a negative, dose dependent impact on working memory (Ilan, Gevins, Coleman, ElSohly, & de Wit, 2005; Silva de Melo et al., 2005). Importantly, these findings suggest that despite acute impairments following cannabis administration, long-term memory function seems to remain unaffected. Furthermore, it appeared that acute cannabis intoxication did not significantly impair working memory in heavy users, unless they were administered high doses of potent cannabis (i.e., high THC content) (Curran, Brignell, Fletcher, Middleton, & Henry, 2002). This divergent effect in experienced users implies that drug tolerance is associated with less impairing side effects.

In contrast to the acute effects, conclusions regarding the long-term impact of chronic cannabis use on working memory are less consistent, due in part to interindividual and intraindividual variability in cannabis use. More recent evidence has suggested that memory deficits persist in individuals who use cannabis frequently and over a long period (for full review see (Schoeler & Bhattacharyya, 2013)). Moreover, early initiation of cannabis use (prior to the age of 17) and strains high in THC, seem to increase the risk of long-term memory dysfunction. These findings are especially troubling given the younger onset of cannabis use as well as recent increases in cannabis potency (Mehmedic et al., 2010).

In line with this research, neurobiological and neuroimaging studies have demonstrated that long-term, early cannabis use lead to the most significant adverse and persistent effects on morphology and brain function, beyond that of acute intoxication (Becker, Wagner, Gouzoulis-Mayfrank, Spuentrup, & Daumann, 2010; Lopez-Larson et al., 2011; Wilson et al., 2000; Yucel et al., 2010). These neuroimaging studies have also found that normal levels of working memory accuracy were associated with a common pattern of
increased overall brain activation, following cannabis use. This enhanced activity in combination with accurate performance suggests neurophysiological inefficiency, whereby increased neural effort is needed in regions normally recruited for task completion. However, it may also suggest that there is a compensatory mechanism involving the recruitment of regions not normally associated with the specific task. Researchers have suggested that this may be indicative of a change in strategy to meet task demands (Bossong, Jager, Bhattacharyya, & Allen, 2014).

One way in which researchers have attempted to better understand the long-term effects of cannabis use on neurophysiological and cognitive function is by employing abstinence paradigms as these studies allow for the assessment of reversibility. In a longitudinal study, Pope and colleagues compared neurocognitive function in heavy cannabis users compared to controls, over 1 month of abstinence. As hypothesized, significant memory impairments in the cannabis-using group on days 0, 1, and 7 were observed; however, the groups were no longer significantly different at day 28, suggesting that the effects of cannabis on memory may not persist following the complete elimination of cannabis from the body. In line with this initial observation, several studies have reported that memory functioning is restored in “regular” cannabis users following abstinence periods ranging from 48 hours to one month (Jager, Kahn, Van Den Brink, Van Ree, & Ramsey, 2006; Schweinsburg et al., 2005; Schweinsburg et al., 2010).

It is important to take into consideration the potential moderating effects of factors in addition to current cannabis use on working memory, including previous exposure to cannabis and additional drugs, frequency and recency of cannabis use, dose of THC administered, THC:CBD ratio, and the particular assessment of memory function. Further
difficulty in interpreting these results lies within the heterogeneity of the selected samples, especially in terms of the criteria used to categorize participants. Previous studies have included groups of former cannabis users, current regular cannabis users, or heavy cannabis users, long-term and short-term cannabis users, or early-onset and late-onset cannabis users (Schoeler & Bhattacharyya, 2013). Overall, it is clear that further research employing diverse methodologies and controlling for confounding variables is necessary for elucidating the acute and long-term effects of cannabis use on working memory.

1.6.5 The Combined Effects of Cannabis Use and Schizophrenia on Working Memory

The relationship between cannabis use and working memory in individuals with schizophrenia has proven to be more complex than was initially assumed, as current research provides inconsistent evidence. D’Souza and colleagues (2005) examined the dose-related effects of intravenous THC using a double blind, randomized, placebo-controlled design in patients with schizophrenia and controls. Compared to those given the placebo, both patients and controls given THC exhibited impaired verbal memory, with more profound deficits observed in the patient group (D’Souza et al., 2005). Similarly, Ringen et al., (2010) evaluated the relationship between acute cannabis use within the last six months and cognitive function including verbal and working memory in 140 patients with schizophrenia. Consistent with previous findings, the researchers found that cannabis use lead to poor verbal learning and working memory performance compared to abstainers (Ringen et al., 2010).

In contrast, there is a growing body of evidence suggesting that chronic cannabis users with schizophrenia may perform better on memory tasks than patients with no history of drug use. Schnell and colleagues (2009) sought to investigate both the enduring effects of
cannabis consumption and the specific patterns of use on cognition. As such, a cognitive test battery was administered to 35 patients with schizophrenia with a lifetime diagnosis of co-morbid cannabis use disorder and 34 patients with no current or past history of cannabis use. Interestingly, cannabis-using patients performed better on the memory tasks, and more frequent cannabis use was associated with better performance, dose-dependently (Schnell, Koethe, Daumann, & Gouzoulis-Mayfrank, 2009). This finding has been replicated in first-episode patients with schizophrenia (Yucel et al., 2012). This body of work was further supported by a longitudinal study conducted by Stirling and colleagues (2005). The researchers found that patients who continued cannabis use during the follow-up period demonstrated a “sparing” of neurocognitive functioning (Stirling, Lewis, Hopkins, & White, 2005). Taken together, these findings outline the divergent and complex effects of acute and chronic cannabis use in this patient population and highlight the need for continued research.

1.7 Overview of Current Study Objectives and Hypotheses

Given the high rates of cannabis abuse and dependence among patients with schizophrenia and the harmful effects of cannabis on illness onset, prognosis and treatment success (D'Souza et al., 2005; Foti et al., 2010; Linszen et al., 1994; Manrique-Garcia et al., 2014), research investigating the neurophysiological effects of cannabis use in patients with schizophrenia is of great importance. Previous neurochemical evidence has suggested that the effects of cannabis on the human brain lie within the complex interaction between the cannabinoid system and inhibitory neuronal networks. More specifically, cannabis acts through the GABAergic system (Eggan & Lewis, 2007), a system vital to inhibitory control and working memory associated gamma oscillations (Bartos et al., 2007; Brown et al., 2007; Fitzgerald et al., 2009). In support of this, previous research has demonstrated that cannabis
impairs cortical inhibition and working memory in otherwise healthy subjects (Ilan et al., 2005; Silva de Melo et al., 2005) and further exacerbates already present inhibitory deficits in patients with schizophrenia (Wobrock et al., 2010). Given that the majority these studies are only conducted cross-sectionally, our understanding is limited to the acute effects of cannabis in these individuals. Thus it is clear that longitudinal studies, employing abstinence paradigms among currently dependent patients, are needed to evaluate the long-term modulatory effects of cannabis on measures of cortical inhibition and working memory. To our knowledge, this is the first study to address the complex relationship between cannabis, cognition, and cortical inhibition in patients with schizophrenia using a within- and between-subjects longitudinal design. The findings of this study may further our understanding of the mechanisms underlying cannabis use among patients will likely help guide novel treatment approaches.

Therefore, the aim of this current study is to examine the neurophysiological effects of cannabis dependence and abstinence in cannabis dependent patients with schizophrenia and non-psychiatric controls. Firstly, at baseline, we will be evaluating the differential effects of cannabis dependence cross-sectionally, on cortical inhibition in patients compared to controls. Through several well-established TMS paradigms, including SICI, LICI, CSP and ICF, we will be able to differentially index GABA_A, GABA_B and NMDA receptor function of the motor cortex. We hypothesize that patients will demonstrate reduced cortical inhibition and excitation indexed through SICI, LICI and ICF measures, as well as a shorter silent period, as assessed through CSP. The second objective of the current study is to evaluate the differential effects of cannabis dependence on working memory performance and gamma oscillatory activity in patients and controls, cross-sectionally, using the N-back task while
EEG is recorded. We hypothesize that patients will demonstrate poorer working memory performance compared to controls. Moreover, we expect to see excessive gamma oscillatory activity specifically in the frontal region in patients with schizophrenia.

As an exploratory analysis, an additional objective of the current study is to evaluate the changes, longitudinally, in cortical inhibition and working memory following a 28-day abstinence period. Thus we will compare TMS measures prior to and following abstinence in the patients and controls who are able to successfully abstain from cannabis use. We hypothesize that following abstinence, both patients and controls will exhibit improved cortical inhibition, with the greatest improvements in patients. Furthermore, we will compare working memory performance and gamma oscillatory activity, prior to and following the cannabis abstinence period. We hypothesize that abstinence will improve working memory performance in both groups compared to baseline, and reduce gamma activity in the patient population.
Chapter 2
Methods

2.1 Study Design

This study is a cross-sectional and longitudinal human laboratory study investigating the effects of cannabis dependence and abstinence on cortical inhibition and working memory associated gamma oscillations in cannabis dependent patients with schizophrenia compared to cannabis dependent non-psychiatric controls. A total of 13 participants (7 controls and 6 patients) completed both the pre and post abstinence TMS testing sessions, while an additional 10 participants (13 controls and 10 patients) completed both working memory testing session (see figure 3.2). The study duration was approximately 4 weeks; this included a screening visit, baseline TMS and working memory visit, and a final TMS working memory test session following the 28-day abstinence period. Included in this abstinence period was weekly TMS testing along with twice-weekly urine drug testing (see figure 2.1). In depth procedural details are provided in section 2.4.
Figure 2.1

Study Design

Figure 2.1: Outline of overall study design including screening assessments along with pre and post TMS and working memory assessments and weekly motor CI measures. Additionally, urine collection took place twice weekly throughout the abstinence period.

This study was approved by the Centre for Addiction and Mental Health’s (CAMH) Research Ethics Board (REB #169/2011) and in accordance to the declaration of Helsinki. Written informed consent was obtained for each participant as approved by the CAMH Research Ethics Board (see Appendix B). Patients with schizophrenia were recruited through CAMH via flyers, word-of-mouth, various outpatient clinics, and referrals. Healthy controls were recruited through flyers, referrals from other studies and online advertisements on Craigslist, and Kijiji.
2.2 Participants

2.2.1 Power analysis

Initially, we aimed to assess cannabis abstinence in 12 patients with schizophrenia and 12 healthy controls, based on an anticipated 25% improvement (Cohen’s $d=0.73$) in cortical inhibition in patients compared to controls at the end of 28 days of cannabis abstinence (with $\alpha=0.05$ and power=80%). Furthermore, an estimated attrition rate of 20% per group is expected resulting in 10 completers for each group.

2.2.2 Sample

All participants were between the ages of 16 to 55. Additionally, participants had to meet criteria for cannabis dependence, as confirmed by the Structured Clinical Interview of the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (SCID for DSM-IV-TR, (APA, 2000a) and could not be treatment seeking for such dependence. Current and lifetime cannabis use and dependence were assessed using several self-report and clinical interview measures. Current cannabinoid levels were assessed using the Narcocheck, a semi quantitative urine drug screen, which detects the presence of cannabis, ranging from 25 to 150 ng/ml. All participants were males, allowing us to focus on the most representative sample of the target population (Goldstein, Seidman, Santangelo, Knapp, & Tsuang, 1994). Further, all participants were current daily cigarette smokers, given that cigarette use is common among cannabis users (Patton, Coffey, Carlin, Sawyer, & Wakefield, 2006) and patients with schizophrenia (Kalman, Morissette, & George, 2005; Lasser et al., 2000), including co-morbid nicotine use was essential. Tobacco smoking status was assessed via self-report and was biochemically verified with expired breath carbon monoxide levels (Vitalograph, Lenexa, KS).
A diagnosis of schizophrenia or schizoaffective disorder was confirmed using the criteria outlined in the SCID for DSM-IV-TR (APA, 2000a). Additionally, inclusion criteria outlined that schizophrenia patients had to be psychiatrically stable, requiring a score <70 on the Positive and Negative Syndrome Scale (Kay, Fiszbein, & Opler, 1987) (PANSS), and on a stable dose of antipsychotic medication for a least one month prior to the time of their assessment. In contrast, healthy controls could not meet SCID for DSM-IV TR criteria for any current or past Axis I psychiatric disorder except for cannabis dependence or past major depression, with at least one year of symptom remission. Additionally, healthy controls could not be taking any psychotropic medications, or have a first-degree relative with psychosis.

Participants were excluded if they met criteria for abuse or dependence for alcohol or any illicit substances within the past 6 months, with the exception of cannabis, nicotine or caffeine. The absence of additional substances was verified using Medtox urine toxicology screens (7-panel-Cannabinoids, Opiates, Amphetamine, Cocaine, Phencyclidine, Barbiturates, and Benzodiazepines). All measures will be discussed in detail in the following section. Additional general exclusion criteria included a history of head injury or loss of consciousness, a neurological or medical condition deemed to affect cognitive function and a personal or first-degree relative with a history of seizures or syncope. Furthermore, all participants had to have a Full Scale IQ ≥ 80 as determined by the WTAR (Wechsler, 2001) and a Test of Memory Malingering (TOMM) score ≥ 45.
2.3 Measures

2.3.1 Clinical interview measures

Structured Clinical Interview for the DSM-IV-TR (SCID-IV)

The SCID-IV (First, 2002) is a semi-structured interview used to diagnose a current or lifetime axis I disorder. In the current study, the SCID was used to verify a diagnosis of schizophrenia or schizoaffective disorder in our patient group and subsequently confirm that the control group did not meet criteria for any axis I disorder. The SCID also allowed for a definitive diagnosis for cannabis dependence in all participants.

Positive and Negative Syndrome Scale (PANSS)

The PANSS (Kay et al., 1987) is a 30-item medical scale used to evaluate symptom severity in individuals with schizophrenia. Through clinical interview, the occurrence and severity of positive symptoms, negative symptoms and general psychopathology are assessed. All items are rated using a 7-point scale (from 1=absent to 7=extreme). The PANSS was chosen due to its high inter-rater reliability (Kay, Opler, & Lindenmayer, 1988) and suitable construct validity (Kay et al., 1987). Administration occurred at screen and weekly throughout the abstinence period.

Addiction Severity Index (ASI)

The ASI (McLellan, Alterman, Cacciola, Metzger, & O'Brien, 1992) is a semi-structured interview used to assess substance abuse and outline the need for new or additional treatment approaches based on the duration, severity and number of symptoms. The ASI evaluates several potential problem areas referencing both the past 30 days and one’s lifetime. These problem domains include psychiatric and medical status, employment and
support, drug and alcohol use, family/social status, and legal status. Using a ten point scale (from 0-1 No real problem, treatment not indicated to 8-9 Extreme problem, treatment absolutely necessary) interviewer severity ratings (ISR) are assigned regarding the severity of problems in each of the seven domains. The ISRs are used to calculate composite scores based on the individuals’ responses corresponding to their experiences in the last 30 days. The ASI was administered at screen in all potential participants.

**Calgary Depression Scale for Schizophrenia (CDSS)**

The CDSS is semi-structured interview, developed to assess the severity of depression symptomatology in individuals with schizophrenia (McLellan et al., 1992). The CDSS is a 9-item measure rated from 0 (absent) to 3 (severe). A total score greater than 4 suggests the presence of minor depression, whereas scores greater than 7 are indicative of major depression. The CDSS was administered at screen in the patient group only.

**2.3.2 Premorbid IQ**

**Wechsler Test of Adult Reading (WTAR)**

The WTAR (Wechsler, 2001) was developed to assess premorbid intelligence. This assessment is comprised of 50 irregularly spelled words, and each correctly pronounced word is given a score of 1. The irregularity of the words makes it difficult to correctly pronounce without having previous knowledge of the words. Thus the participant’s vocabulary, and by extension IQ, can be assessed. Raw scores were standardized by age.
2.3.3 Extrapyramidal Side Effects

Abnormal Involuntary Movement Scale (AIMS)

The AIMS (Guy & Cleary, 1976) was designed to measure dyskinetic symptoms, a common side effect associated with long-term antipsychotic treatment in patients with schizophrenia. The AIMS allows for early detection and ongoing surveillance of tardive dyskinesia (TD). This 12-item assessment of involuntary movements is rated on a 5-point scale ranging from 0 (none) to 4 (severe), except for items 11 and 12 (dental care) which are answered with either a “yes” or a “no”. A score of 2 or higher, i.e. mild movements in two categories or moderate movements in one area, (Barnes, 1989) suggest the presence of TD.

Barnes Akathisia Rating Scale (BARS)

The BARS (Barnes, 1989) is used to assess neuroleptic-induced akathisia, a syndrome of motor restlessness, in individuals with schizophrenia. The BARS incorporates subjective reports of awareness and distress related to akathisia, along with objective motor restlessness observations rated using a 4-point scale. Finally, global clinical assessment is rated using a 5-point scale.

Simpson Angus Rating Scale (SARS)

The SARS (Simpson & Angus, 1970) is used to assess neuroleptic-induced parkinsonism, evaluating gait (hypokinesia), rigidity and glabella tap, as well as tremors and salivation. The SARS is a 10-item measurement rated on a 4-point scale.
2.3.4 Substance Use Measures

The Alcohol Use Disorders Identification Test (AUDIT)

The AUDIT (Saunders, Aasland, Babor, de la Fuente, & Grant, 1993) was developed by the World Health Organization to quickly screen for excessive alcohol use. The ten-item measure assesses alcohol consumption, alcohol dependence and other alcohol related problems. Men who achieve a score of 8 or more (7 for women) out of a possible score of 40, likely partake in hazardous or harmful drinking behaviours, whereas a score of 20 or more is indicative of alcohol dependence.

Timeline Follow Back (TLFB)

The TLFB (Sobell, Sobell, Leo, & Cancilla, 1988) is used as an estimate of alcohol, marijuana, tobacco and caffeine use over a one-week period. Participants were asked to retrospectively assess the frequency of substance use on a day-to-day basis. Given the importance of cannabis use in this study specifically, participants were also asked to estimate the number of times they had purchased cannabis in the last week, as well as the number or grams and amount of money spent on cannabis.

Fagerstrom Test of Nicotine Dependence (FTND)

The FTND (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) is one of the most commonly used measures, developed to assess self-reported nicotine dependence. This brief 6-item questionnaire ranges in scores from 0 to 10, where higher scores correspond to more severe nicotine dependence. Importantly, the FTND has been shown to have adequate reliability in smokers with and without schizophrenia (Weinberger et al., 2007).
Joint Years

Joint years was used as a measure of cumulative exposure to cannabis. This measure was adapted from a commonly used measure in nicotine research called cigarette years. A joint-year was defined as smoking on average one joint per day for 1 year (e.g., 1 joint year = 365 joints smoked in one year).

Urine Drug Screen

The Narcocheck is a semi-quantitative urine drug-screening test that has 5 levels of cannabis detection ranging from 25 to 150 ng/ml. This test is able to detect marijuana, hashish and cannabis resin present in the users urine. In this study, the Narcocheck was used once weekly throughout the abstinence period to assess for changes in cannabis using behaviours and verify abstinence at day 28.

2.3.5 Cortical Inhibition

Participants were instructed to abstain from cannabis 12 hours prior to the TMS session; however, ad libitum cigarette use was allowed throughout the test session to avoid nicotine withdrawal. During testing, participants were seated in a comfortable chair with their arms supported passively by a pillow, and were asked to maintain relaxation throughout the session. Surface EMG was recorded from the abductor pollicis brevis (APB) muscle of the right hand with disposable disc electrodes placed in a tendon-belly arrangement over the bulk of the muscle. An additional metal ground electrode was placed on the inside of the forearm distal to the wrist. EMG was monitored on a computer screen and the signal was amplified (Intronix Technologies Corporation Model 2024F, Bolton, ON, Canada), filtered (band pass
2 Hz–2.5 kHz), digitized at 5 kHz (Micro 1401, Cambridge Electronics Design, Cambridge, UK) and stored in a laboratory computer for offline analysis.

TMS was applied to the hand area of the left motor cortex with a 7 cm figure-of-eight magnetic coil and two Magstim 200 magnetic stimulators (Magstim, Whitland, Dyfed, Wales). The coil was held tangentially on the head with the handle pointing backward midline at a 45° lateral angle. The optimal coil position was determined for each subject individually and was marked to ensure consistent positioning. The RMT was defined as the minimal intensity that produced a MEP of >50 μV in 5 of 10 trials in relaxed the APB muscle (Kujirai et al., 1993). The output intensity required to elicit a MEP 1 mV peak to peak was determined (Kujirai et al., 1993).

SICI and ICF were assessed using a standard paired pulse procedure (Kujirai et al., 1993). A subthreshold conditioning stimulus (CS), which was set at 80% of RMT, preceded a suprathreshold test stimulus (TS). The TS was delivered at an intensity that produced an average 1mV peak-to-peak amplitude (Kujirai et al., 1993). The CS was applied to the motor cortex before the TS at one of five randomized ISIs: 2 ms and 4 ms for SICI and 10 ms, 15 ms, and 20 ms for ICF along with the TS alone. Seventy-two trials were performed, 12 for each condition. Changes in the TS MEP amplitude at each ISI were expressed as a percentage of the mean unconditioned MEP amplitude (Daskalakis, Christensen, Fitzgerald, & Chen, 2002).

For LICI, a suprathreshold CS preceded a suprathreshold TS (set at the adjusted 1 mv peak-to-peak) at one of three randomized ISIs: 100 ms, 150 ms and 200 ms. Forty trials were performed, 10 for each ISI as well as 10 for the TS alone. Finally, the measurement of the
CSP duration was obtained in moderately tonically active APB muscle (i.e., 20% of maximum contraction) by stimulating the motor cortex with an intensity of 140% of the RMT. Ten trials were performed. CSP duration was measured as the time from the MEP onset to the return of any voluntary EMG activity, referred to as the absolute CSP. The CSP was determined with our previously published automated method (Daskalakis et al., 2003). Using CFSview in CED Signal 2.0, the 10 trials were averaged off-line and CSP duration was measured on an averaged recording. The absolute CSP value was calculated as the time of offset of the period of EMG activity suppression minus the time of onset. The order of administration of SICI/ICF, LICI and CSP were counterbalanced for each participant (refer to figure 2.2).
Figure 2.2

Motor Cortical Inhibition Paradigms

Figure 2.2. Adapted from (Barr, Fitzgerald, Farzan, George, & Daskalakis, 2008). EMG recordings following: A) SICI a conditioning stimulus (CS) precedes the TS by 2–5 ms and inhibits the corresponding MEP B) LICI, the CS precedes the TS by approximately 100–200 ms and inhibits the corresponding MEP C) CSP induced following a 140% suprathreshold TS while the right hand muscle is tonically activated D) ICF a conditioning stimulus (CS) precedes the TS by 10-20 sec.
2.3.6 Working Memory

N-Back Task

Subjects performed the N-back task while EEG activity was recorded (STIM2, Neuroscan, U.S.A.). Black capital letters were presented on a computer monitor one at a time in a continuous sequence. Participants were instructed to respond by pushing a button (2; target) if the present stimulus was identical to the stimulus presented N (1 or 3) trials back; otherwise, participants pushed a different button (1; non-target). Each letter was present on the screen for 250 ms, followed by a 3000 ms time frame to respond. Stimuli were presented for 15 minutes in the 1-back condition and 30 minutes in the 3-back, ensuring that there were a satisfactory number of correct responses for data analysis (refer to figure 2.3). The number of target letters in each condition was: 46 of 198 (23.2%) in the 1-back and 59 of 400 trials (14.6%) in the 3-back condition. Outcome measures included task accuracy and response time. The order of conditions were randomized and counterbalanced across subjects to prevent order effects.

EEG Measurement of Induced γ Oscillatory Activity

Induced oscillatory responses are non-phase-locked to the stimulus onset with a latency within the first 500 ms of stimulus onset, which varied trial-by-trial (Tallon-Baudry, Kreiter, & Bertrand, 1999). In contrast to induced activity, evoked oscillatory activity is phase-locked to the stimulus onset, with a fixed latency within 100 ms of the stimulus onset (Tallon-Baudry et al., 1999). Although both evoked and induced gamma activities are associated with working memory (Barr et al., 2008; Basar-Eroglu et al., 2007; Howard et al., 2003), it has been suggested that induced gamma band activity may be more closely related to the maintenance of information in working memory, whereas evoked oscillations are
considered less related to higher cognitive functions (Tallon-Baudry et al., 1999). Several studies have demonstrated that evoked oscillations are more strongly influenced by attention (Tiitinen et al., 1993) and task instructions (Hermann, 1993). As such, we measured mean induced gamma power from frontal electrodes (Fpz, Fp1, Fp2, AFz, AF3, AF4, AF7, AF8, Fz, F1, F2, F3, F4, F5, F6, F7, F8) while subjects completed the N-back task.

**Figure 2.3**

N-Back Task

![Diagram of N-Back Task](image)

**Figure 2.3.** Adapted from (Barr et al., 2013)  
*A) An example of the 1- and 3-back conditions that were completed by patients and controls. Participants were asked to push a button if the current letter was identical to the letter presented N trials back and push a different button for the non-targets. Only correct responses for target (TC) were included in the data analysis.  
B) The timing of one trial for a total time of 3000 ms including the presentation of a letter separated by a + sign followed by a subsequent letter. The data gamma power were measured following the presentation of the stimulus (250 ms) until 750 ms.*
EEG Recording

EEG data were acquired using a 64-electrode cap and Synamps2 DC-coupled EEG system (Compumedics, U.S.A.). Data were 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 the mastoid electrodes, TP7 and TP8.

Offline EEG Processing

EEG recordings were processed offline using MATLAB (The MathWorks Inc. Natick, MA, USA) and EEGLAB toolbox (Delorme reference). Data were down sampled to 1 kHz and filtered by using second order, Butterworth, zero-phase shift 1–55 Hz band pass filter. Second, EEG data was segmented -1400 ms to +3100 ms relative to the stimulus onset. Next, a channels-by-trials matrix of all ones was created and assigned the value to zero if an epoch had: (1) amplitude larger than +/- 150 µV; (2) power spectrum that violated 1/f power law; or (3) standard deviation 3 times larger than average of all trials. A channel was rejected if its corresponding row had more than 60% of columns (i.e., trials) coded as zeros and an epoch was removed if its corresponding column had more than 20% of rows (i.e., channels) coded as zeros. Epochs were then manually inspected and trials containing irregularities were removed. Finally, an independent component analysis (ICA) (EEGLAB toolbox; Infomax algorithm) was performed to remove eye-blink traces, muscle artifacts, and other noise from the EEG data.

Gamma power was analyzed for the target trials that were responded to correctly across all N-back conditions. The raw data was filtered for gamma (30-50 Hz) frequency ranges with zero-phase shift. We then calculated the time series for gamma amplitude using the Hilbert transform. Additionally, we created concatenated signal of 5000 ms using
randomly selected epochs for each subject, each trial type, and at each electrode, as this corresponds with induced gamma power.

2.3.7 Cannabis Abstinence Paradigm

Abstinence was assessed weekly through the administration of NarcoCheck. However, to our knowledge this test has not been measured in a clinical research setting and thus the NarcoCheck was only used to capture decreasing THC levels or alternatively any sudden increases throughout the 28-day abstinence period. End-point abstinence was assessed using MEDTOX urine toxicology. Abstinent individuals were those who did not use cannabis within the 28-day period and demonstrated endpoint detection THC-COOH levels below 50ng/ml, which is the accepted cut-off level in the literature (Huestis, Mitchell, & Cone, 1995). If participants did not meet these criteria, they were classified as relapsers or non-abstainers. All stored urine from those classified as relapses was sent to our CAMH clinical laboratory and was further analyzed by gas chromatography-mass spectroscopy to obtain quantitative THC-COOH and creatinine concentrations. This analysis confirmed that relapsers did in fact use cannabis over the abstinence period.

Contingency management and brief behavioural therapy were administered weekly to aid in cannabis abstinence. The basic model of contingency management promotes behavioural change through the administration of positive or negative contingencies. In the current study, we used a cost-effective strategy called the “fishbowl” technique whereby participants who demonstrated decreasing THC levels through urinalysis, were able to select a slip of paper from a fish bowl. The bowl will contain 250 slips of paper, half of which were nonwinning slips that said Sorry, try again while the winning slips could be exchanged for prizes that varied in value. Of the winning slips, 109 were small prizes consisting of $1-5
coupons to local restaurants, 15 were large prizes, worth up to a maximum of $20 in value and 1 of the slips was a jumbo prize valued at $100. In addition to the weekly contingency management, on day 29, participants received a $3000 bonus if urine results were indicative of cannabis abstinence.

Furthermore, individual weekly therapy sessions were administered on days 1, 8, 15 and 22 in order to aid in study attendance and abstinence. A combination of psychoeducation, motivational interviewing, coping skills therapy, and cognitive-behavioural relapse prevention were implemented, given research suggesting that a combination of interventions leads to the highest rates of abstinence success (Budney et al., 2000). Early sessions focused on building a rapport and provided psychoeducation in order to heighten awareness of the personal consequences associated with cannabis use. Additionally, motivation interviewing was used to increase participants’ willingness to stop using. Finally, later sessions focused on coping strategies and relapse prevention techniques. These therapy sessions lasted approximately 30 minutes and were conducted by trained research assistants in the CAMH Schizophrenia Program.

2.4 Study Procedure

Once potential participants were identified, a telephone screen was completed, which briefly assessed eligibility based on current and past medical and substance use. Those who were deemed eligible were invited into the Biobehavioural Addictions and Concurrent Disorders Research Laboratory (BACRDL; Principal Investigator: Dr. Tony P. George, M.D., FRCPC) at CAMH for an additional screening assessment. Upon arrival for a screening assessment, participants were asked to complete the informed consent as well as a post-consent quiz to verify their understanding of the consent. Following this, demographic
information, IQ testing, as well as measures of depression (HAM-D), drug use (ASI, TLFB, AUDIT), carbon monoxide levels and Medtox™ urine toxicology were administered. For schizophrenia patients, clinical assessments were conducted including the SCID-IV, PANSS, with the AIMS, SARS, and BARS. The candidate, Michelle Goodman conducted screening measures along with Rachel Rabin, who primarily conducted the SCID, PANSS, and ASI assessments.

Data presented are a subset of a larger study, therefore only baseline and post abstinence testing sessions will be included. All testing sessions were conducted at the Temerty Centre for Therapeutic Intervention, CAMH. Participants were instructed to abstain from using cannabis 12 hours prior to baseline testing. During the baseline visit, motor cortical inhibition was administered including SICI, LICI, ICF and CSP. Following this, N-Back EEG was collected for the 1 and 3-Back conditions. This testing session was repeated following the 28-day abstinence period. Participants were compensated for their time at a rate of $10 per hour, which they received at the end of every testing session, after approximately 3 hours.

2.5 Data Analysis

2.5.1 Study Sample

Independent t-tests and chi-squared tests were used to analyze differences between the two diagnostic groups on demographic variables including age, years of education, IQ, daily cannabis and cigarette use and joint years.
2.5.2 Cortical Inhibition

All data were analyzed using the Statistical Program for Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, Ill). All tests were two-tailed and the level of significance was set at $\alpha = 0.05$. Separate repeated measures ANOVAs were conducted for SICI, LICI and ICF with diagnosis (patients versus controls) as the between subjects factor and ISI (SICI ISI: 2 ms and 4 ms, ICF ISI: 10 ms, 15 ms and 20 ms, LICI ISI: 100 ms, 150 ms and 200 ms) as the within subjects factor. Independent t-tests were conducted for RMT and CSP. Post-hoc tests were conducted where appropriate with an adjusted Bonferroni corrected alpha level to account for multiple comparisons. Pearson correlation analyses were conducted on each of the TMS paradigms and demographic variables, and were analyzed within groups when significant.

2.5.3 Working Memory

Baseline working memory performance (accuracy and reaction time) and gamma oscillatory activity were analyzed using separate repeated measures ANOVAs with diagnosis (schizophrenia vs. healthy controls) as the between-subjects factor and N-back load (1- and 3-back) as the within-subjects factor. Pearson correlation analyses were conducted on performance and oscillatory activity on the 1-back and 3-back condition and demographic variables, and were analyzed within groups when significant.
Chapter 3
Results

3.1 Baseline Results

3.1.1 Demographics

A total sample of 26 individuals participated in the baseline testing session of this study, recruited over a 24-month period. Twelve of these participants met diagnostic criteria for schizophrenia or schizoaffective disorder and fourteen were non-psychiatric healthy controls. Of these participants, all completed baseline N-Back testing. However, 3 patients with schizophrenia and 5 healthy controls failed to meet eligibility for TMS testing due to history of concussions and other head injuries, history of seizures, as well as 1 control participant who did not want to take part in this portion of the study (refer to figure 3.2).

All patients were clinically stable, with PANSS scores below 70. Ten of these patients were on atypical antipsychotic medications including: risperidone (4), quetiapine (3) olanzapine (1), clozapine (1), and paliperidone (1). Furthermore, 1 patient was on the typical antipsychotics flupentixol, and 1 patient received both fluphenazine and quetiapine. In addition to antipsychotic medications, several patients were on antidepressants for symptoms of depression or anxiety including escitalopram, lorazepam and citalopram and trazodone. Finally, 1 of these patients was on the anticholinergic medication benzotropine as well as several additional cholesterol and diabetes medications including metformin, nicotinic acid, atorvastatin, indapamide, and glyburide.
Given that sample sizes differed between cortical inhibition and working memory assessments, separate demographic tables were presented for our TMS and working memory findings. The following demographics included all baseline participants who completed the working memory assessment (for baseline TMS demographics refer to table 3.1). As expected, group differences were revealed on measures of IQ as measured by the WTAR, with higher average IQ scores in the control group (t(25) = -3.061, p = .005). Group differences were revealed on years of education, with fewer years seen in the patient group (t(25) = -2.116, p = .044). Importantly, there were no significant differences in daily cigarette use (p = .520), daily cannabis use (p = .971) and joint years (p = .157) (refer to table 3.3). Interestingly, IQ was negatively correlated with joint years (r(25) = -0.393, p = .043). This correlation only remained significant when all participants were included.

Figure 3.1. Significant negative correlation between IQ and joint years r (25) = -0.393, p = .043, Thus higher IQ was associated with fewer joint years.
Figure 3.2
Consort Diagram for patients with schizophrenia (SZ) and healthy controls (HC)

Assessed for eligibility via phone screen (N=123)

Initially eligible (N=71)

Excluded (n = 47):
Concurrent Substance use or Axis I,
PANSS > 70,
FSIQ < 80, or
Negative for THC

Total sample (N= 26)

Baseline N-Back sample
SZ= 12
HC= 14

Baseline TMS sample
SZ= 9
HC= 10

Completed N-Back sample
SZ= 9
HC= 13

Completed TMS sample
SZ= 6
HC= 7
3.1.2 Baseline TMS Assessments

Table 3.1

Baseline TMS demographics, clinical means, and standard deviations by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>HC (N = 10)</th>
<th>SZ (N = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.54 ± 6.98</td>
<td>30.33 ± 9.63</td>
<td>.955</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>70% (7)</td>
<td>11.1% (1)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>20% (2)</td>
<td>66.7% (6)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10% (1)</td>
<td>22.2% (2)</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>12.3 ± 2.00</td>
<td>11.0 ± 2.18</td>
<td>.191</td>
</tr>
<tr>
<td>IQ</td>
<td>99.54 ± 9.43</td>
<td>91.67 ± 1.01</td>
<td>.089</td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>-</td>
<td>13.78 ± 2.95</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>-</td>
<td>11.89 ± 2.71</td>
<td></td>
</tr>
<tr>
<td>general</td>
<td>-</td>
<td>27.44 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>-</td>
<td>53.11 ± 8.31</td>
<td></td>
</tr>
<tr>
<td>CPZ equivalent (mg/day)</td>
<td>-</td>
<td>531.92 ± 262.65</td>
<td></td>
</tr>
<tr>
<td>CPD</td>
<td>11.99 ± 1.02</td>
<td>9.52 ± 5.91</td>
<td>.592</td>
</tr>
<tr>
<td>GPD</td>
<td>1.86 ± 1.27</td>
<td>1.30 ± .88</td>
<td>.276</td>
</tr>
<tr>
<td>Joint years</td>
<td>8.75 ± 4.8</td>
<td>12.35 ± 9.36</td>
<td>.281</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

HC = Healthy controls, SZ = patient with schizophrenia CPZ = chlorpromazine, CPD = cigarettes per day, GPD = grams per day

3.1.2.1 Baseline Inhibitory Assessments: SICI, LICI, and CSP

To evaluate baseline differences in GABA_A inhibitory activity between patients and controls (SICI), a repeated measures ANOVA was conducted with ISI (2 ms versus 4 ms) as the within-subjects variable and diagnosis (patients and controls) as the between-subjects variable. This analysis revealed a significant main effect of ISI (F(1,17) = 9.244, p = .007) (see figure 3.3). A paired samples t-test revealed greater inhibition at 2 ms compared to 4 ms (t(18) = 3.123, p =0.006). No significant ISI x diagnosis interaction was found (p = .888),
however there was a trend towards a significant diagnosis effect ($F(1,17) = 3.473, p = 0.080$), with greater inhibition in patients.

![Graph showing baseline short interval cortical inhibition (SICI) between patients (SZ) and healthy controls (HC) across 2 ms (SICI2) and 4 ms (SICI4) ISIs. Results revealed a significant main effect of ISI $F(1,17) = 9.244, p = .007$. Bars represent (±) 1 standard deviation. Asterisks (*) represent significance.](image)

**Figure 3.3.** Baseline short interval cortical inhibition (SICI) between patients (SZ) and healthy controls (HC) across 2 ms (SICI2) and 4 ms (SICI4) ISIs. Results revealed a significant main effect of ISI $F(1,17) = 9.244, p = .007$. Bars represent (±) 1 standard deviation. Asterisks (*) represent significance.

To evaluate baseline differences in GABA$_B$ inhibitory activity between patients and controls measured through LICI, a repeated measures ANOVA was conducted with ISI (100 ms versus 150 ms versus 200 ms) as the within-subjects variable and diagnosis (patients versus controls) as the between-subjects variable. Results revealed a significant main effect of ISI ($F(2,38) = 8.876, p = .001$) (see figure 3.4). A paired samples t-test found significant differences between 150 and 200 ms ($t(20) = 3.180, p = 0.005$) and 100 and 200 ms ($t(2) = 2.366, p = 0.028$). Only the first comparison remained significant following an adjustment for multiple comparisons (Bonferroni correction) with greater inhibition in the 150 ms condition. No significant diagnosis effect was found ($p = .928$), however a trend towards a significant ISI x diagnosis interaction ($F(2,38) = 2.778, p = 0.075$) was revealed.
**Figure 3.4.** Baseline long interval cortical inhibition (LICI) between patients (SZ) and healthy controls (HC) across 100 ms (LICI100), 150 ms (LICI150) and 200 (LICI200). Results revealed a significant main effect of ISI ($F(2,38) = 8.876, p = .001$). Bars represent ($\pm$) 1 standard deviation. Asterisks (*) represent significance.
An additional measure of GABA$_B$ inhibitory activity was assessed using an independent t-test with CSP as the test variable and diagnosis as the grouping variable. Results revealed significantly longer baseline CSP in patients ($M = 0.167, SD = 0.030$) compared to controls ($M = 0.135, SD = 0.010$) ($t(18) = 2.257, p = 0.037$).

**Figure 3.5.** Baseline cortical silent period (CSP) for patients (SZ) and healthy controls (HC). Significant differences were found between diagnosis regarding the lengths of silent periods ($t(18) = 2.257, p = 0.037$). Bars represent (±) 1 standard deviation. Asterisks (*) represent significance.
3.1.2.2 Baseline Excitatory Assessments: RMT and ICF

To examine potential baseline differences in cortical excitatory activity between patients and controls, we conducted an independent t-test on RMT. Results revealed no significant diagnostic differences ($p = 0.207$). Additionally, a repeated measures ANOVA was conducted on ICF to assess NMDA cortical excitation. There were also no significant differences in ICF between controls and patients ($p = 0.278$); however, a large effect size was revealed for the 20 ms interval (Cohen’s $d = .951$; refer to table 3.2).

**Figure 3.6.** Baseline cortical facilitation (ICF) between patients (SZ) and healthy controls (HC) across 10 ms (ICF10), 15 ms (ICF15) and 20 ms (ICF20). No significant differences were found at any ISI. Bars represent ($\pm$) 1 standard deviation.
Table 3.2
*Cohen’s D and p-values for TMS Measures*

<table>
<thead>
<tr>
<th>TMS Measure</th>
<th>ISI</th>
<th>HC (N = 10)</th>
<th>SZ (N = 9)</th>
<th>Cohen’s d</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SICI</td>
<td>2 ms</td>
<td>29.5 ± 62.8</td>
<td>72.2 ± 24.0</td>
<td>.915</td>
<td>.065</td>
</tr>
<tr>
<td></td>
<td>4 ms</td>
<td>14.9 ± 70.3</td>
<td>54.1 ± 27.5</td>
<td>.794</td>
<td>.109</td>
</tr>
<tr>
<td>LICI</td>
<td>100 ms</td>
<td>58.3 ± 43.1</td>
<td>58.2 ± 70.4</td>
<td>.065</td>
<td>.881</td>
</tr>
<tr>
<td></td>
<td>150 ms</td>
<td>61.4 ± 38.9</td>
<td>67.6 ± 63.9</td>
<td>.177</td>
<td>.683</td>
</tr>
<tr>
<td></td>
<td>200 ms</td>
<td>50.2 ± 39.3</td>
<td>24.0 ± 66.2</td>
<td>.342</td>
<td>.429</td>
</tr>
<tr>
<td>CSP</td>
<td>-</td>
<td>0.13 ± 0.03</td>
<td>0.16 ± 0.03</td>
<td>.926</td>
<td>.037</td>
</tr>
<tr>
<td>ICF</td>
<td>10 ms</td>
<td>130.2 ± 64.7</td>
<td>148.2 ± 57.8</td>
<td>.198</td>
<td>.109</td>
</tr>
<tr>
<td></td>
<td>15 ms</td>
<td>128.4 ± 54.1</td>
<td>150.7 ± 52.7</td>
<td>.336</td>
<td>.668</td>
</tr>
<tr>
<td></td>
<td>20 ms</td>
<td>103.3 ± 44.8</td>
<td>147.9 ± 51.1</td>
<td>.951</td>
<td>.054</td>
</tr>
</tbody>
</table>

**Bolded values indicate a medium to large effect size (> .5)**

Percent inhibition or facilitation are expressed as mean ± SD. Cohen’s D values and p-values comparing short interval cortical inhibition (SICI), long interval cortical inhibition (LICI), cortical silent period (CSP) and intracortical facilitation (ICF) between patients (SZ) and healthy controls (HC). Bolded results indicate that there is a strong or significant effect for SICI (2 ms and 4 ms ISI), CSP and ICF (20ms ISI).
3.1.3 Baseline Working Memory Assessments

Table 3.3
Baseline N-Back demographics, clinical means, and standard deviations by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>HC (N = 14)</th>
<th>SZ (N = 12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.93 ± 6.62</td>
<td>32.00 ± 1.02</td>
<td>.353</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>78.6% (11)</td>
<td>25.1% (3)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>14.3% (2)</td>
<td>50.0% (6)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7.1% (1)</td>
<td>16.7% (2)</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.1 ± 2.67</td>
<td>11.13 ± 2.02</td>
<td>.044*</td>
</tr>
<tr>
<td>IQ</td>
<td>102.40 ± 9.43</td>
<td>91.17 ± 9.37</td>
<td>.005*</td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td>13.67 ± 3.06</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td>11.58 ± 2.61</td>
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<tr>
<td>general</td>
<td></td>
<td>27.00 ± 4.77</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>52.25 ± 9.01</td>
<td></td>
</tr>
<tr>
<td>CPZ equivalents (mg/day)</td>
<td></td>
<td>463.21 ± 302.06</td>
<td></td>
</tr>
<tr>
<td>CPD</td>
<td>10.43 ± 9.05</td>
<td>12.56 ± 7.55</td>
<td>.520</td>
</tr>
<tr>
<td>GPD</td>
<td>1.55 ± 1.20</td>
<td>1.53 ± .89</td>
<td>.971</td>
</tr>
<tr>
<td>Joint years</td>
<td>7.94 ± 4.50</td>
<td>11.68 ± 8.60</td>
<td>.157</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD
Results that are significant at the 0.05 level are marked with an (*)
HC = healthy controls, SZ = patients with schizophrenia, CPZ = chlorpromazine, CPD = cigarettes per day, GPD = grams per day

3.1.3.1 Baseline Working Memory Performance

In total, 12 patients with schizophrenia and 14 healthy controls were included in our baseline analysis. As expected, a repeated measures ANOVA revealed a main effect of N-back load, between the 1-back condition ($M = 85.45$, $SD = 12.09$) and the 3-back condition ($M = 49.67$, $SD = 23.09$) ($F(1,24) = 97.021$, $p > 0.001$). Both groups demonstrated significantly lower scores on the 3-back, as measured by a paired t-test ($t (25) = 10.019$, $p < 0.001$)(refer to figure 3.7). No significant N-back load x diagnosis interaction was found ($p =$
0.695). No significant effect of diagnosis was revealed ($p = 0.186$). A repeated measures ANOVA on reaction time (ms) found a main effect of load, between the 1-back condition ($M = 821.23, SD = 166.76$) and the 3-back condition ($M = 1041.40, SD = 258.80$) ($F(1,24) = 23.835, p > 0.001$). As expected, paired samples t-test revealed longer reaction times in the 3-back condition ($t(25) = -5.056, p < 0.001$).

![Figure 3.7. Baseline N-back performance between patients (SZ) and healthy controls (HC) and the 1-back and 3-back conditions. Performance is significantly lower in the 3-back condition across both groups $F(1,24) = 23.835, p > 0.001$. No performance differences were found in the 1-back condition or 3-back condition between groups. Bars represent (±) 1 standard deviation. Asterisks (*) represent significance.](image)

### 3.1.3.2 Baseline Gamma Power

To evaluate baseline gamma oscillatory activity elicited during a working memory task, a repeated measures ANOVA was conducted for the frontal electrodes encompassing the DLPFC (Fpz, Fp1, Fp2, AFz, AF3, AF4, AF7, AF8, Fz, F1, F2, F3, F4, F5, F6, F7, F8) and for the target correct responses only, with N-back condition (1 and 3-back) as the within-
Subjects variable and diagnosis (patients versus controls) as the between-subjects variable.

Results did not reveal a significant main effect of memory load ($p = .589$) nor did we find a significant N-back load x diagnosis interaction ($p = .390$). Similarly no significant effect of diagnosis was revealed ($p = .823$).

**Figure 3.8.** Mean gamma (30-50 Hz) oscillatory power from target correct (TC) responses elicited across 1-back and 3-back conditions in patients (SZ) compared to healthy controls (HC). Bar graphs represent mean gamma power across the frontal electrodes (Fpz, Fp1, Fp2, AFz, AF3, AF4, AF7, AF8, Fz, F1, F2, F3, F4, F5, F6, F7, F8). Bars represent ($\pm$) 1 standard deviation. Topographical illustration of mean gamma power elicited during 1- and 3-back in patients with SCZ compared to HS. Greater gamma power is represented by hot or red colours.
3.2 Exploratory Longitudinal Results

The following section is exploratory given that this study is too underpowered to assess the effects of cannabis abstinence in patients and controls. Abstinent individuals were defined as those who did not use cannabis within the 28-day period and who demonstrated endpoint THC-COOH levels below 50ng/ml (for more details refer to section 2.3.7). Urinalysis was conducted for most of the participants in the study and reported as normalized-creatinine THC-COOH levels. THC-COOH is the main non-psychoactive metabolite detected in the urine of cannabis users. Normalizing THC-COOH concentrations to urinary creatinine controls for individual variability in hydration and urine output (Lafolie et al., 1991). It should be noted that at baseline, healthy control abstainers had higher levels of THC-COOH as compared to patient abstainers. Regardless of diagnosis, at baseline, relapsers demonstrated higher average THC-COOH levels than the abstainer.

Table 3.4
Creatinine-normalized THC-COOH levels in patient and control abstainers and relapsers

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>Baseline THCCOOH (ng/ml)</th>
<th>Post THCCOOH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstainers</td>
<td>HC (N = 7)</td>
<td>77.68 ± 110.70</td>
<td>2.83 ± 5.40</td>
</tr>
<tr>
<td></td>
<td>SZ (N = 3)</td>
<td>21.9 ± 9.53</td>
<td>1.13 ± 1.19</td>
</tr>
<tr>
<td>Relapsers</td>
<td>HC (N = 2)</td>
<td>157.35 ± 68.93</td>
<td>127.45 ± 130.74</td>
</tr>
<tr>
<td></td>
<td>SZ (N = 3)</td>
<td>86.9 ± 66.93</td>
<td>50.17 ± 78.2</td>
</tr>
</tbody>
</table>

Urinalysis of creatinine-normalized TH-CCOOH levels prior to and following a 28-day abstinence period in patients (SZ) and healthy controls (HC). THC-COOH is the main non-psychoactive metabolite, detected in the urine of cannabis users. Values above 50ng/ml are representative of “relapse” or new cannabis use.
3.2.1 Longitudinal TMS Assessments

Six patients with schizophrenia and 7 controls participated in the abstinence TMS testing session. Of the patients who participated, 4 successfully abstained, while 2 relapsed and for the controls, 3 successfully abstained and 4 relapsed (see Table 3.4).

Table 3.5

Post TMS demographics, clinical means, and standard deviations of the sample by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>HC abst (N = 3)</th>
<th>HC non-abst (N = 4)</th>
<th>SZ abst (N = 4)</th>
<th>SZ non-abst (N = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.0 ± 5.57</td>
<td>32.0 ± 6.16</td>
<td>32.75 ± 13.2</td>
<td>26.5 ± .707</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
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<td>66.7% (2)</td>
<td>66.7% (2)</td>
<td>-</td>
<td>50% (1)</td>
</tr>
<tr>
<td>African American</td>
<td>-</td>
<td>33.3% (1)</td>
<td>75% (3)</td>
<td>50% (1)</td>
</tr>
<tr>
<td>Other</td>
<td>33.3% (1)</td>
<td>-</td>
<td>25% (1)</td>
<td>-</td>
</tr>
<tr>
<td>Education (years)</td>
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<td>12.0 ± 1.41</td>
<td>12.75 ± 1.5</td>
<td>11.0 ± 1.41</td>
</tr>
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<td>IQ</td>
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<td>95.75 ± 8.90</td>
<td>92.25 ± 11.4</td>
<td>92.0 ± 5.6</td>
</tr>
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<td>PANSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-</td>
<td>14.75 ± 3.10</td>
<td>14.5 ± 4.95</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>11.0 ± 2.83</td>
<td>11.0 ± 2.83</td>
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<tr>
<td>general</td>
<td>-</td>
<td>-</td>
<td>29.5 ± 4.20</td>
<td>25.0 ± 5.66</td>
</tr>
<tr>
<td>total</td>
<td>-</td>
<td>-</td>
<td>55.25 ± 9.29</td>
<td>50.5 ± 13.0</td>
</tr>
<tr>
<td>CPZ equivalents (mg/day)</td>
<td>-</td>
<td>-</td>
<td>549.7 ± 4111.9</td>
<td>250 ± 353.5</td>
</tr>
<tr>
<td>CPD</td>
<td>18.86 ± 18.41</td>
<td>9.43 ± 6.31</td>
<td>7.22 ± 3.96</td>
<td>16.0 ± 5.6</td>
</tr>
<tr>
<td>GPD</td>
<td>1.83 ± 1.56</td>
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<td>.748 ± .227</td>
<td>2.31 ± 1.60</td>
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<tr>
<td>Joint years</td>
<td>12.11 ± 4.99</td>
<td>10.35 ± 3.15</td>
<td>13.65 ± 13.12</td>
<td>9.66 ± 4.73</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

HC = healthy controls, SZ = patients with schizophrenia
Abst = abstainer and non-abst = relapse
CPZ = chlorpromazine, CPD = cigarettes per day, GPD = grams per day

3.2.1.1 Longitudinal Inhibitory Assessments: SICI, LICI, and CSP

We aimed to evaluate the potential longitudinal changes in GABA\textsubscript{A} inhibitory activity through SICI. We compared the reduction in cortical inhibition at baseline and following 28
days of abstinence in patients with schizophrenia and healthy controls. Furthermore, we separately graphed patients and controls who successfully abstained (figure 3.9 A) from those who relapsed (figure 3.9 B).

**Figure 3.9.** Longitudinal data for *short interval cortical inhibition (SICI)* between patients (SZ) and healthy controls (HC) across 2 ms (SICI2) and 4 ms (SICI4) ISIs at baseline and at following 28-days of abstinence. A represents a graph of abstainer HC and SZ, B represents a graph of relapser HC and SZ. Bars represent (± 1 standard deviation).

To evaluate longitudinal changes in GABA\textsubscript{B} inhibitory activity, we administered LICI and CSP in patients and controls. We compared the reduction in cortical inhibition (figure 3.10) as well as the change in silent period length (figure 3.11) at baseline and
following 28 days of abstinence separately in patients and controls who successfully abstained (figure 3.10 A, 3.11A) and those who relapsed (figure 3.10 B, 3.11 B).

**Figure 3.10.** Longitudinal data comparing long interval cortical inhibition (LICI) between patients (SZ) and healthy controls (HC) across 100 ms (LICI100), 150 ms (LICI150) and 200 ms (LICI200) at baseline and following 28-days of abstinence. A represents a graph of abstainer HC and SZ, B represents a graph of relapser HC and SZ. Bars represent (±) 1 standard deviation.
Figure 3.11. Longitudinal data comparing cortical silent period (CSP) between patients (SZ) and healthy controls at baseline and following 28-days of abstinence. A represents a graph of abstainer HC and SZ, B represents a graph of relaper HC and SZ. Bars represent ($\pm$) 1 standard deviation.

3.2.1.2 Longitudinal Excitatory Assessment: ICF

We also evaluated the potential longitudinal changes in NMDA excitatory activity through ICF. We compared the enhancement in cortical excitation at baseline and following 28 days of abstinence in patients with schizophrenia and healthy controls. We separately
graphed patients and controls who successfully abstained (figure 3.12 A) from those who relapsed (figure 3.12 B).

**Figure 3.12.** Longitudinal data comparing intracortical facilitation (ICF) between patients (SZ) and healthy controls (HC) across 10 ms (ICF10), 15 ms (ICF15) and 20 ms (ICF20) at baseline and following 28-day abstinence. A represents a graph of abstainer HC and SZ, B represents a graph of relaper HC and SZ. Bars represent (±) 1 standard deviation.
3.2.2 Longitudinal Working Memory Assessments

Ten patients with schizophrenia and 13 controls participated in the longitudinal TMS testing session. Of the patients who participated, 6 successfully abstained while 4 relapsed, and for the controls, 8 successfully abstained and 5 relapsed (see Table 3.5).

Table 3.6
Post WM demographics, clinical means, and (±) 1 standard deviations by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>HC abst (N = 8)</th>
<th>HC non-abst (N = 5)</th>
<th>SZ abst (N = 6)</th>
<th>SZ non-abst (N = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.25 ± 5.20</td>
<td>31.2 ± 5.63</td>
<td>32.2 ± 11.50</td>
<td>33.5 ± 11.79</td>
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<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>87.5% (7)</td>
<td>66.7% (2)</td>
<td>16.7% (1)</td>
<td>50% (1)</td>
</tr>
<tr>
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<td>33.3% (1)</td>
<td>50% (3)</td>
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<tr>
<td>Other</td>
<td>33.3% (1)</td>
<td>-</td>
<td>16.7% (1)</td>
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</tr>
<tr>
<td>Education (years)</td>
<td>12.5 ± 3.35</td>
<td>12.8 ± 2.17</td>
<td>12.90 ± 1.34</td>
<td>10.75 ± 0.57</td>
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<td>IQ</td>
<td>105.88 ± 8.03</td>
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<td>90.6 ± 10.57</td>
<td>92.25 ± 6.24</td>
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<td></td>
</tr>
<tr>
<td>positive</td>
<td>-</td>
<td>-</td>
<td>14.0 ± 3.16</td>
<td>14.5 ± 4.04</td>
</tr>
<tr>
<td>negative</td>
<td>-</td>
<td>-</td>
<td>10.4 ± 2.79</td>
<td>11.5 ± 1.91</td>
</tr>
<tr>
<td>general</td>
<td>-</td>
<td>-</td>
<td>28.0 ± 4.95</td>
<td>26.25 ± 5.74</td>
</tr>
<tr>
<td>total</td>
<td>-</td>
<td>-</td>
<td>55.4 ± 10.26</td>
<td>52.25 ± 11.44</td>
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<td>CPZ equivalents (mg/day)</td>
<td>-</td>
<td>-</td>
<td>651.33 ± 238.1</td>
<td>412.5 ± 287.2</td>
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<tr>
<td>CPD</td>
<td>10.93 ± 11.89</td>
<td>9.03 ± 5.54</td>
<td>10.77 ± 8.66</td>
<td>18.0 ± 4.0</td>
</tr>
<tr>
<td>GPD</td>
<td>1.24 ± .998</td>
<td>2.39 ± 1.37</td>
<td>1.13 ± .869</td>
<td>2.17 ± .98</td>
</tr>
<tr>
<td>Joint years</td>
<td>7.03 ± 5.35</td>
<td>8.99 ± 4.08</td>
<td>11.81 ± 12.07</td>
<td>10.97 ± 5.33</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD
HC = healthy controls and SZ = patients with schizophrenia
Abst = abstainer and non-abst = relapse
CPZ = chlorpromazine, CPD = cigarettes per day, GPD = grams per day
3.2.2.1 Longitudinal Working Memory Performance

The N-back task was administered at baseline and following 28 days of cannabis abstinence in patient and control abstainers and relapers. Accuracy for target correct responses on the 1 and 3-back conditions were graphed separately.

**Figure 3.13.** Longitudinal N-back performance between patients (SZ) and healthy controls (HC) abstainers and relapers at baseline and following 28-day abstinence on the 1-back and 3-back conditions. Bars represent (±) 1 standard deviation.
3.2.2.2 Longitudinal Gamma Power

Mean gamma oscillatory power was measured at baseline and following abstinence in patient and control abstainers and relapers. Topographs illustrated the mean gamma power elicited during the 1- and 3-back conditions.

**Mean Gamma 1-Back (µV2)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Abstinence</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td><img src="HC_1-Back.png" alt="" /></td>
<td><img src="HC_Abstinence.png" alt="" /></td>
<td><img src="HC_Relapse.png" alt="" /></td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>SZ</td>
<td><img src="SZ_1-Back.png" alt="" /></td>
<td><img src="SZ_Abstinence.png" alt="" /></td>
<td><img src="SZ_Relapse.png" alt="" /></td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

**Mean Gamma 3-Back (µV2)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Abstinence</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td><img src="HC_3-Back.png" alt="" /></td>
<td><img src="HC_Abstinence.png" alt="" /></td>
<td><img src="HC_Relapse.png" alt="" /></td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>SZ</td>
<td><img src="SZ_3-Back.png" alt="" /></td>
<td><img src="SZ_Abstinence.png" alt="" /></td>
<td><img src="SZ_Relapse.png" alt="" /></td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure 3.14.** Topographical illustration of mean gamma power elicited during 1- and 3-back in patients (SZ) and healthy control (HC). Included are topo plots at baseline and following a 28-day abstinence period in abstainers and relapers. Greater gamma power is represented by hot or red colours.
Chapter 4
Discussion

4.1 General Discussion

Given the high rates of cannabis abuse and dependence among patients with schizophrenia and the detrimental effects of cannabis on illness onset, prognosis and treatment success (D'Souza et al., 2005; Foti et al., 2010; Linszen et al., 1994; Manrique-Garcia et al., 2014), uncovering the mechanism underlying this common co-morbidity is of great value. As such, the aim of this study was to investigate the neurophysiological effects of cannabis dependence in patients with schizophrenia and non-psychiatric controls. To our knowledge, this was the first study to address the complex relationship between cannabis, cognition, and cortical inhibition in patients with schizophrenia using a within- and between-subjects longitudinal design. Given that cannabis inhibits GABAergic neurotransmission, which is necessary for both cortical inhibition and working memory, we hypothesized that cannabis dependence would further impair GABAergic deficits present in patients with schizophrenia. We assessed GABA inhibitory neurotransmission through several well-established TMS paradigms as well as indirectly through working memory performance and gamma oscillatory activity. As an exploratory secondary analysis, we evaluated longitudinal changes in cortical inhibition and working memory following a 28-day cannabis abstinence period. We hypothesized that cortical inhibition and working memory deficits would be potentiated with cannabis abstinence in both patients and non-psychiatric controls.

The main finding in the current study was greater GABA_B mediated inhibitory neurotransmission, as measured by CSP, in cannabis dependent patients compared to dependent healthy controls. In regards to SICI, while no significant effect of diagnosis was
found, due in part to the large within-group variability, our results suggest that cannabis dependent patients may have more GABA\(\alpha\) mediated inhibition compared to controls. No significant differences were found on LICI or on our measures of cortical excitation, including ICF and RMT. With regards to our working memory findings, results suggest that patient and control cannabis users perform similarly on the N-back task, along with comparable levels of frontal gamma oscillatory activity. Taken together, these results suggest that cannabis dependence has differential effects on cortical inhibition in controls compared to patients with schizophrenia. While these contradict our initial hypotheses, this preliminary research provides novel insight into the mechanisms underlying co-morbid cannabis use among individuals with schizophrenia.

4.1.1 Study Sample

In the current study, our patient group demonstrated a significantly lower IQ and fewer years of education as compared to the control group. This finding is in accordance with previous cross-sectional studies both in patients with schizophrenia (Kremen, Seidman, Faraone, & Tsuang, 2001) and in chronic cannabis users (Arseneault, Cannon, Witton, & Murray, 2004); therefore, this sample seems to be representative of the target population. Additionally, all participants were male. While this does limit the generalizability of the study, cannabis use has been shown to be especially common in young males experiencing their first-episode of psychosis (Koskinen et al., 2010).

Of note, this was one of the first studies conducted in cannabis dependent individuals, to employ a longitudinal abstinence paradigm, with twice-weekly urinalysis. Additionally, we accounted for important confounding variables associated with substance use. This
included, frequency and recency of use as well as the amount of money spent weekly on cannabis, which is thought to indirectly index of the quality of cannabis consumed. Furthermore, unlike previous research in this field, we implemented a clear definition of cannabis dependence, whereby all participants had to be daily cannabis users for a minimum of 1 year. Additionally, participants had to meet DSM-IV criteria for cannabis dependence and could not currently consume or meet dependence for, any additional illicit substances within the past 6 months. Finally, we also controlled for co-morbid cigarette use unlike previous research, given its common co-occurrence in this population (Kalman et al., 2005; Patton et al., 2006). Importantly, daily cannabis and cigarette consumption did not differ between controls and patients.

4.1.2 TMS Findings

The main finding in the current study was increased GABA$_{B}$ mediated inhibitory control, as measured by CSP, in cannabis dependent patients compared to dependent healthy controls. This finding contradicts the previous two studies investigating the effects of cannabis use on cortical inhibition in first episode patients with schizophrenia (Wobrock et al., 2010) and controls (Fitzgerald et al., 2009), as neither study found significant differences on this measure. CSP is considered to be one of the more robust and consistent TMS measures; however recent observations have brought to light large variability between studies in terms of the effects of schizophrenia on CSP. For example, early research demonstrated GABA$_{B}$ specific CSP deficits in patients with schizophrenia (Eichhammer et al., 2004; Fitzgerald, Brown, et al., 2002a). However, consistent with the current study, more recent research has found a lengthening of the CSP length in patients with schizophrenia (Hasan et al., 2012; Strube et al., 2014; Wobrock et al., 2009).
In comparison to previously published CSP values, our cannabis dependent controls and patients displayed both longer than “average” (Hasan et al., 2012) and shorter than “average” (Strube et al., 2014) CSP lengths. Therefore, in the context of previous research, this suggests that no clear unidirectional comparisons regarding the effects of cannabis use on cortical silent period in patients or controls can be drawn. Methodological differences, including severity and duration of illness, stimulation intensity and the effects of medication, likely contribute to these divergent findings. For example, stimulation intensity in both of these previous studies was set to the participant’s 1mV (which is typically 120% of their RMT), whereas in the current study, stimulation intensity was set to 140% of the RMT (Hasan et al., 2012; Strube et al., 2014). Additionally, clozapine has been shown to significantly lengthen CSP in patients with schizophrenia (Daskalakis, Christensen, et al., 2008). In the current study, we included one patient who was treated with clozapine, and given that no outliers were removed from analysis, we cannot rule out the possibility that this participant is driving the current finding. In summary, our results suggest that cannabis dependent patients with schizophrenia exhibit greater GABA\textsubscript{B} mediated inhibition compared to cannabis dependent controls.

Regarding our SICI findings, unlike previous research and contrary to our hypothesis, we did not find reduced SICI in cannabis dependent patients. In fact, with a trend towards a significant diagnosis effect, as well as a large effect size, our results suggest the opposite effect of cannabis on GABA\textsubscript{A} mediated cortical inhibition, with enhanced inhibition in patients. This large effect size yet lack of statistical significance is likely due to small sample sizes and large variability within groups. Interestingly, when compared to the heavy using dependent controls in Fitzgerald’s study, our current control group demonstrated similarly
low levels of GABA<sub>A</sub> mediated cortical inhibition (Fitzgerald et al., 2009). Additionally, when compared to previously published values regarding the percentage of inhibition in Fitzgerald’s healthy, non-cannabis using sample, controls in the current study demonstrate lower levels of inhibition (Fitzgerald et al., 2009).

In contrast, when compared to Wobrock’s previously published SICI findings in first-episode cannabis-using patients, cannabis dependent patients in the current study demonstrated a greater amount of cortical inhibition. Similarly, when compared to published findings in both recent onset and chronically ill non–using patients with schizophrenia (Strube et al., 2014), cannabis dependent patients in the current study also demonstrated more cortical inhibition. Of note, these comparisons must be interpreted with caution, given that they were made across studies, which introduces a number of uncontrolled variables. For example, in Wobrock’s study, dependence was assessed with the European Addiction Severity Index as well as the DSM-IV criteria of substance abuse/dependence, however, these cannabis users only had to use weekly and more than 20 times in their lifetime. Given such divergence in cannabis dependence criteria, it is not possible to draw direct comparisons regarding cortical inhibitory results from the current study with that of the previous findings.

In the context of previously published data (Fitzgerald et al., 2009; Strube et al., 2014; Wobrock et al., 2010), these SICI results suggest that cannabis may reduce GABA<sub>A</sub> inhibition in controls whereas cannabis may have the opposite effect on inhibition in patients. In support of this, our group recently demonstrated this finding with preliminary data. This data showed that cannabis using patients had more cortical inhibition as measured by SICI compared to non-using patients, whereas control cannabis users demonstrated significantly
less inhibition as compared to non-using controls (Goodman, 2015). While the previous studies mentioned above all assessed SICI, these findings must be considered with caution, given that differences within these SICI paradigms, participant pool and study designs likely exist. Thus, these comparisons go beyond the scope of the current project.

In line with previous findings in cannabis using controls compared to non-users (Fitzgerald et al., 2009) as well as in medicated and unmedicated patients compared to controls (Fitzgerald et al., 2003), no LICI diagnostic differences were revealed in the current study. Given that both LICI and CSP are thought to index GABA\textsubscript{B} mediated inhibition, and significant differences in CSP were observed in the current study, it would suggest that LICI might not be sensitive enough to detect these potential diagnostic differences. These divergent effects may also be due to the differential mechanisms underlying these TMS paradigms. For example, CSP assesses the duration of inhibition, whereas LICI indexes the magnitude of inhibition at a single time point (McDonnell, Orekhov, & Ziemann, 2006). Furthermore, CSP has been shown to be associated with inhibition at the level of the spinal cord, whereas long interval inhibition may take place more so at the level of the cortex (Chen, Lozano, & Ashby, 1999).

While no significant diagnostic differences were observed on measures of cortical excitation including RMT and ICF, a large effect size at 20 milliseconds for ICF was revealed. The previous study conducted by Wobrock and colleagues (2010) found enhanced ICF in cannabis dependent first-episode patients with schizophrenia (Wobrock et al., 2010). In the current study, we found a trend towards higher facilitation in the cannabis dependent patient group, although these differences were not significant. It has been suggested that enhanced ICF in these patients might best be explained by an increased glutamatergic input
along with disturbances in cholinergic intracortical neuronal circuits (Wobrock et al., 2010). While no study has looked specifically at the effects of chronic cannabis use on cortical facilitation, previous research has shown that nicotine, alcohol and cocaine users demonstrate cortical hypoxcitability (Barr et al., 2008; Boutros et al., 2005; Conte et al., 2008; Lang, Hasan, Sueske, Paulus, & Nitsche, 2008). Given that these previous studies were conducted in otherwise healthy controls, we cannot rule out the possibility that these divergent findings may be due, in part, to the underlying brain abnormalities in NMDA receptor functioning seen in patients with schizophrenia (Falkai, Wobrock, Schneider-Axmann, & Gruber, 2008). Additionally, several of these previous studies only examined the acute drug effects on cortical facilitation and did not employ the appropriate abstinence period, thus testing participants while intoxicated. As such, it is clear that continued research investigating the effects of cannabis use on cortical excitability is necessary.

In summary, these findings suggest that cannabis has differential effects in controls and patients in terms of GABA\textsubscript{B} inhibitory functioning, demonstrated through a significantly longer CSP in patients. The large effect sizes and trends towards significant diagnostic effects on measures of GABA\textsubscript{A} and NMDA, suggest that with a larger sample size, these differences may become more evident. While these findings oppose our initial hypotheses, they depict an interesting picture of the neurophysiological effects of cannabis dependence among patients with schizophrenia. Given the paucity of research in this field, it is clear that further research confirming this finding is necessary.
4.1.3 Working Memory Findings

Contrary to our initial hypothesis, cannabis dependent patients with schizophrenia performed as well as cannabis dependent controls on both the 1 and 3-back conditions of the N-back verbal working memory task. Previous research has suggested that patients with schizophrenia consistently perform worse than controls on working memory tasks. In fact, working memory deficits are among the most replicable and robust findings in this population (Forbes et al., 2009). Comparisons have been drawn between the cognitive impairments seen in patients with schizophrenia with that of cannabis users; these cognitive domains included attention, learning and inhibition (Radhakrishnan et al., 2014; Solowij & Michie, 2007). Thus it would follow that cannabis use among patients with schizophrenia would exacerbate already present working memory deficits. However, there is a growing body of evidence suggesting that chronic cannabis users with schizophrenia perform better on memory tasks than patients with no history of drug use (Rabin et al., 2011; Schnell et al., 2009; Stirling et al., 2005; Yucel et al., 2012). Thus, in order to determine exactly how cannabis dependence impacts working memory deficits in patients with schizophrenia, the current sample must be compared to a non-dependent patient sample.

Current research has suggested that under low working memory load conditions (i.e., the 1-back), accuracy in patients and controls is comparable; however, increased memory load was associated with a deterioration in patients’ performance (Barr et al., 2013; Carter et al., 1998). Unfortunately, few studies have administered the most challenging 3-back condition to patients with schizophrenia. In one study conducted by Barr et al. (2013), researchers aimed to evaluate the effects of a non-invasive brain stimulation technique called repetitive transcranial magnetic stimulation (rTMS) on working memory performance in
schizophrenia patients. Prior to and following sham stimulation, 3-back accuracy slightly decreased from 36 to 33 percent (Barr et al., 2013). In an earlier study conducted by Jansma et al. (2004), researchers found that half of their patient sample performed near chance on the 3-back condition, with scores lower than 35 percent (Jansma, Ramsey, van der Wee, & Kahn, 2004). In contrast, cannabis dependent patients in the current study scored above average at 82 percent on the 1-back and 44 percent on the 3-back. It should be noted that in the current study, no outliers were removed, thus we included one extreme outlier who performed well below chance on the 3-back condition. Excluding this patient’s data increased 3-back performance to 49 percent. Taken together, these findings suggest that cannabis dependent patients may perform better on the 3-back condition than non-using patients.

Similarly to the cognitive deficits observed in non-using patients with schizophrenia, converging neuroimaging, neurobiological and longitudinal research has suggested that memory deficits persist in otherwise healthy individuals who use cannabis at an early age, frequently, and over a long period of time (Becker et al., 2010; Lopez-Larson et al., 2011; Mehmedic et al., 2010; Yucel et al., 2010). To our knowledge, only one study has administered the 3-back condition in cannabis using non-psychiatric controls. In this previous study, N-back accuracy was compared among daily cannabis users (>7 joints per week for a minimum duration of 3 years) (Verdejo-Garcia et al., 2013). Unlike the current study, researchers reported probability of hit (i.e., number of hits/number of hits+number of misses). The 1-back probability of hit was 91 percent in cannabis users, while their 3-back probability of hit was significantly lower in at 61 percent. These scores were similar to, yet slightly better than that of the cannabis dependent controls in the current study, who scored an 89 percent on the 1-back and a 54 percent on the 3-back. It should be noted that the
accuracy from these two studies cannot be directly compared, given the different methods of reporting performance (i.e., % accuracy versus probability of hit) and number of available target correct responses. Unlike the current study, the cannabis-using group had to abstain for 72 hours, which coincides with the height of cannabis withdrawal symptoms (Budney, Moore, Vandrey, & Hughes, 2003); however, these withdrawal symptoms were medically controlled (Verdejo-Garcia et al., 2013). Thus these slight differences in performance could be due to the period of cannabis abstinence, as well as the divergent methods of reporting performance.

Compared to non-using controls, cannabis dependent controls in the current study seemed to perform worse on the more challenging 3-back condition. In healthy controls, 3-back accuracy has been shown to range from above 80% (Prilipko et al., 2011; Sanchez-Carrion et al., 2008) to approximately 70% (Barr et al., 2010; Rose & Ebmeier, 2006). Therefore, these results suggest that cannabis dependence in healthy controls impairs working memory performance as compared to non-using controls. This phenomenon is generally supported in the literature, whereby memory deficits persist beyond acute intoxication in chronic users (for full review see (Schoeler & Bhattacharyya, 2013)).

Regarding the effects of cannabis dependence on baseline gamma oscillatory activity, we initially hypothesized that cannabis dependence among patients would further exacerbate their excessive gamma activity. However, our results revealed that patients and controls exhibited similarly high level of gamma activity in the prefrontal cortex. One study conducted by our group revealed excessive oscillations in patients compared to controls during the 3-back condition (Barr et al., 2010). The researchers suggested that this finding may be due in part to GABAergic deficits, specifically through the action of GABA$_B$, ...
ultimately leading to a lack of inhibition of gamma oscillations (Farzan et al., 2010a). In the current study, a similar lack of modulation was observed in both controls and patients, given that gamma oscillatory activity was as high in the 1-back condition as the 3-back. This suggests that cannabis may have similar underlying impairing effects on neural oscillations as those seen in patients with schizophrenia. Specifically this lack of gamma modulation may be due, in part to GABA$_B$ dysfunction, which was observed among our control cannabis using participants.

Similarly to the excessive oscillatory activity among patients, cannabis use has been shown to increase gamma oscillations in healthy controls. The CB1R receptor is primarily located on GABAergic inhibitory interneurons, and exogenous activation of these receptors through THC, for example, suppresses GABA (Freund et al., 2003). Thus, it has been suggested that cannabis may increase neuronal synchrony similarly to that of a GABA receptor antagonist. These antagonists have been shown to lead to convulsive seizures (Steriade, Amzica, Neckelmann, & Timofeev, 1998), due to excessive or hypersynchronous neuronal activity in the brain (Shusterman & Troy, 2008). Moreover, models of epilepsy utilize cannabinoids due to their pro-convulsant side effects (Clement, Hawkins, Lichtman, & Cravatt, 2003). In line with this research, cannabis dependent controls in the current study demonstrated increased levels of gamma oscillatory activity compared to previously published results in non-using controls (Barr et al., 2010). Thus, it is possible that the lack of diagnostic differences in gamma activity in the current study could be attributed to excessive oscillations both in control cannabis users and patients with schizophrenia.

Thus taken together, these findings suggest that aberrant gamma oscillatory activity may underlie cognitive deficits associated with both schizophrenia and cannabis use. Based
on our working memory performance results, it may seem as though there are no differential diagnostic effects of cannabis dependence, however the potential effects of cannabis become clearer when compared to previously published 3-back accuracy data conducted in a group of non-cannabis using patients and controls. As such, these findings suggest that cannabis use may be more impairing in control users, where as cannabis dependent patients with schizophrenia seem to performed better than non-using patients. Given that this is the first study evaluating working memory performance combined with gamma activity in this population, it is clear that further research is warranted.

4.1.4 Exploratory Findings

To date, all studies that have investigated the effects of cannabis either on cortical inhibition or working memory associated oscillatory activity in patients with schizophrenia have employed cross-sectional designs. As such, the current study aimed to investigate the longitudinal neurophysiological effects of cannabis throughout a 28-day cannabis abstinence period. Employing an abstinence paradigm allows for the evaluation of the long-term effects of cannabis, beyond that of acute intoxication and withdrawal. Given that cannabis likely differentially modulates cortical functioning in patients and controls, we directly compared these effects in cannabis dependent individuals with and without schizophrenia. Additionally, within each of these groups, valuable information was also drawn from comparing those who successfully abstained and those who relapsed.

In the current study, the small sample size limited our ability to draw conclusions regarding the longitudinal effects of cannabis abstinence. This was especially true for our TMS findings, given the large within group variability and lack of clear or consistent inhibitory patterns. However, there is one interesting preliminary LICI finding in abstaining
patients versus those who relapsed. Results revealed an increase in GABA$_B$ mediated inhibition following 28-day abstinence in patients who successfully abstained. In contrast, the reverse was found in patients who relapsed, whereby, on day 28, relapsers demonstrated a decrease in inhibition. While it is an interesting finding, it is clear that continued research is needed in order to validate this potential pattern with a greater number of participants.

Regarding the working memory findings, although subtle, results revealed a decrease in accuracy on both the 1 and 3-back conditions among patient abstainers. In line with this finding, these patients also demonstrated slight decreases in frontal gamma oscillatory activity. While this finding may suggest that patient’s optimal level of gamma activity and accuracy is present during acute cannabis abstinence (i.e. at baseline), continued research is needed, as these findings are very preliminary. An additional interesting finding within the 3-back condition in both patients and controls, was that those who successfully abstained from cannabis performed better at baseline than those who went on to relapse (refer to figure 3.13). This may suggest that N-back accuracy, or more broadly cognitive functioning, may predict one’s ability to successfully abstain from cannabis. While this relationship has never been studied in cannabis users, in cigarette smokers with schizophrenia, poor working memory performance has been shown to predict relapse (Dolan et al., 2004). These preliminary findings highlight the need for continued research on the effects of both cannabis dependence and abstinence in order to better understand the long-term neurophysiological impact of cannabis on the brain.

4.1.5 Potential Mechanisms

While the exact mechanisms underlying the effects of cannabis in individuals with schizophrenia are not well understood, overlapping neurocognitive and neurophysiological
research suggests that interferences in the homeostatic role of the eCB system may underlie this common co-morbidity. The eCB system acts through synaptic retrograde signalling to ultimately control the release of other neurotransmitters, including GABA, glutamate and dopamine (Schlicker and Kathmann, 2001). Activation of the CB1 receptor, through either its endogenous ligands anandamide and 2-AG, or exogenous cannabis use, mediates the production and release of inhibitory and excitatory neurotransmitters key to cortical inhibition, higher order cognitive functions and synaptic plasticity (i.e., learning and memory) (Chevaleyre, Takahashi, & Castillo, 2006; Howlett et al., 2004; Piomelli, 2003). Exposure to exogenous cannabinoids, especially during adolescence, has been shown to significantly disrupt the protective effect of the eCB system (James, James, & Thwaites, 2013). Chronic cannabis use leading to the development of dependence and tolerance has been shown to alter the functionality of the eCB system; however the exact neurochemical and neurophysiological effects of chronic cannabis use are still largely unknown (Hoffman, Oz, Caulder, & Lupica, 2003; Sim-Selley, 2003). Given that cannabis is known to suppress GABAergic inhibitory neurotransmission (Iversen, 2003) we hypothesized that, cannabis dependence would lead to deficits in cortical inhibition. In line with this hypothesis, cannabis dependent controls in the current study demonstrated less GABA_B mediated cortical inhibition when compared to cannabis dependent patients with schizophrenia.

Interest in the involvement of the eCB system underlying cognitive processes was motivated by evidence demonstrating that CB1 receptors are highly expressed in the hippocampus (Herkenham, 1991), a brain region vital to learning and memory. Evidence has consistently demonstrated that cannabinoids manipulate the various stages of learning and memory, including acquisition, consolidation, and retrieval (Ilan et al., 2005; Silva de Melo
et al., 2005). These behavioural effects are thought to be produced, in part, by an imbalance in GABAergic neuronal transmission in the PFC (Pistis et al., 2002). As such, we hypothesized that cannabis dependence in patients and controls would increase gamma oscillatory activity and lead to poorer working memory performance. Based on previously published 3-back accuracy results in healthy controls (Barr et al., 2010), we found that cannabis dependent controls in the current study demonstrated lower working memory accuracy as well as high frontal gamma oscillatory activity. Thus we propose that these cortical inhibitory and working memory deficits seen in non-psychiatric cannabis dependent controls are likely due to the suppressing effects of cannabis use on GABA inhibitory neurotransmission.

Regarding the patient sample, we hypothesized that cannabis dependence would further exacerbate GABAergic deficits, leading to aberrant cortical inhibition and poor working memory performance. However, our results revealed that cannabis dependent patients actually demonstrated improved cortical inhibition and similar working memory performance to our control group. Thus this implies that chronic cannabis use does not necessarily have the same neurophysiological effects in controls and patients and highlights the complex relationship between schizophrenia and cannabis dependence. There are a number of plausible reasons as to why we did not confirm our hypotheses. Firstly, there is large body of evidence illustrating alterations in the eCB system in patients with schizophrenia, regardless of cannabis consumption. For example, post-mortem studies in patients demonstrated an increased density of CB1 receptors in DLPFC (Dean et al., 2001) and anterior cingulate (Zavitsanou, Garrick, & Huang, 2004) as well as elevated levels of anandamide in the cerebrospinal fluid (CSF) in acute schizophrenia (Giuffrida et al., 2004).
Thus these pre-existing eCB alterations likely influence the “normal” effects of cannabis use among patients.

Secondly, it is possible that patients develop a compensatory mechanism in response to these underlying eCB system deficits. This may therefore account for the improved cortical inhibition and working memory performance in cannabis dependent patients observed in the current study. The existence of a compensatory mechanism in patients has previously been suggested. For example, one study found that in the DLPFC of patients with schizophrenia, GABA\textsubscript{A} receptors were up-regulated at pyramidal axons, which was thought to develop in response to deficient GABA release from chandelier axon terminals (Jensen, Chiu, Sokolova, Lester, & Mody, 2003). Researchers have interpreted this finding to represent a compensatory response to GABA deficits (Lewis et al., 2005). Similarly, Bossong and colleagues proposed that chronic cannabis use might lead to a compensatory mechanism, involving the recruitment of regions not normally associated with a working memory task (Bossong et al., 2014). Thus these findings suggest that chronic cannabis using patients develop a GABA specific compensatory mechanism, which may in part, account for our current unexpected findings in patients.

An additional potential reason as to why cannabis dependent patients demonstrated better cortical inhibition and working memory performance compared to non-using patients is drawn from social cognitive research. Several researchers have suggested that cannabis-using patients with schizophrenia may represent a subset individuals with this disorder, following the observation that these patients reliably performed better on cognitive tasks than non-using patients (Rodriguez-Sanchez et al., 2010; Yucel et al., 2012). It has been suggested that cannabis-using patients may be cognitively less vulnerable given that their onset of
psychosis may not have developed without the initiation of cannabis use. These individuals may therefore represent a ‘healthier’ group (Arndt, Tyrrell, Flaum, & Andreasen, 1992), who are able to lead the complex life of a drug user (Joyal, Halle, Lapierre, & Hodgin(s), 2003; Rodriguez-Sanchez et al., 2010). Given that these cannabis-using patients may have better premorbid social and cognitive functioning, it is possible that they may not have as profound GABAergic deficits as their non-using counterparts. In line with this hypothesis, preliminary data from our group have demonstrated that cannabis dependent patients have better GABA<sub>A</sub> mediated cortical inhibition than non-cannabis dependent patients (Goodman, 2015). Therefore, the current findings of enhanced cortical inhibition and working memory performance in cannabis dependent patients may provide preliminary support for the hypothesis that patients who present with co-morbid cannabis use represent a subset of the patient population with better premorbid social functioning may.

The fact that this GABAergic mechanism cannot adequately account for all of the observed findings in patients and controls highlights the complex neurophysiological interactions between mental illness and drug use. Of note, this current mechanism does not take into consideration the widespread effects of cannabis on dopamine, glutamate, and other neurotransmitters. Similar to the pathophysiological theories underlying schizophrenia, deficits in one neurotransmitter alone may not sufficiently account for this complex co-morbidity. Furthermore, the majority of neurochemical and neurophysiological research is based on the acute effects of cannabis among patients with schizophrenia and many unknowns still exist regarding the long-term effects of chronic cannabis use. Thus, without a better understanding of these unknowns, we are limited in our interpretation of the underlying neurophysiological and neurochemical mechanisms.
4.2 Limitations

These results should be interpreted in light of several limitations. One of the most noticeable weaknesses in the current study was the small sample size. This was especially true for our longitudinal analysis, given that our small sample was further subdivided into abstainers and relapses. Nevertheless, the 3-back working memory load condition may capture the divergent neurophysiological effects in abstainers versus relapsers with continued testing. With such a small sample size, we chose not to remove outliers from the study, which likely contributed to the large variability seen within our TMS measures. An additional and significant limitation was the lack of a non cannabis-using control group, for both our patients and controls. This lack of a true control group likely masked the true neurophysiological effects of cannabis use.

Although our patient and control groups were well matched in terms of age, cigarette use as well as daily and lifetime cannabis use, there were several limitations within our sample. Firstly, the study included only male participants, thus reducing the generalizability of our findings. A key reason why only males were selected for this study was due to previous research suggesting that cannabis is especially common in young males experiencing their first-episode of psychosis (Koskinen et al., 2010). Thus, these findings highlight the difficulties in recruiting cannabis dependent female patients. Further differences in our sample were observed in the breakdown of racial backgrounds between our two groups, with a higher percentage of Caucasian controls and non-Caucasian patients. While these differences were not significant, it is possible that cannabis metabolism may be influenced by race. To date, no studies have looked into this specifically in cannabis users however, research has suggested that the pharmacodynamics and pharmacokinetics of other
drugs vary based on race (Chen, 2006).

Secondly, while we collected data regarding current and past patterns of cannabis use, we did not differentiate between the strains of cannabis nor did we chemically verify the potency of cannabis used by our participants. It has been well-documented that the neuropharmacological effects of cannabis vary widely across the different strains due to the varying ratios of the cannabinoid constituents (Potter, Clark, & Brown, 2008). It should be noted, however, that other than the joint year and IQ negative correlation, we did not find a significant correlation between the TMS or working memory assessments and cannabis consumption, including grams per day and joint years.

Thirdly, it should be considered that while participants were excluded if they met criteria for other substance abuse or dependence 6 months prior to testing, several participants had a past diagnosis of an alcohol or substance use disorder. Lifetime substance dependence has been shown to have long-term effects on neurocognition (Potter, Downey, & Stough, 2013; Verdejo-Garcia, Bechara, Recknor, & Perez-Garcia, 2006) and cortical functioning (Bolla et al., 2003; Ersche et al., 2005) even following the complete elimination of the substance from the body. Additionally, all participants were current cigarette users, given that nicotine dependence commonly co-occurs in cannabis users (Patton et al., 2006) and patients with schizophrenia (Kalman et al., 2005; Lasser et al., 2000). In patients, nicotine use is known to selectively enhance neurocognitive function (Sacco et al., 2005), thus the presence of nicotine in our participants likely confounds our working memory performance results and potentially our neurophysiological findings. Importantly, in the current study, the frequency of past substance dependence and daily cigarette use did not significantly differ between patients and controls. However, in order to truly elucidate the
effects of cannabis, future studies should better control for these confounding variables.

Additionally, in the patient group specifically, it should be noted that antipsychotic medications pose as a considerable confounding factor (Davey et al., 1997). Differences in RMT have been found in patients treated with risperidone compared to those on olanzapine (Fitzgerald, Brown, Daskalakis, & Kulkarni, 2002b). Similarly, for paired-pulse measurements, studies have shown that clozapine significantly lengthens CSP (Daskalakis, Christensen, et al., 2008) whereas benzodiazepines alter GABA specific TMS paradigms (Di Lazzaro et al., 2008). Furthermore, these medications likely influenced our working memory findings. For example, studies have shown enhanced 40 Hz oscillatory activity (Hong et al., 2004) and cognitive performance (Bilder et al., 2002; Meltzer & McGurk, 1999; Sharma, Hughes, Soni, & Kumari, 2003) in patients on second-generation antipsychotics. In response to these findings, we did not include any patient on benzodiazepines for the TMS portion of the study; however, participants on a variety of antipsychotics were included and therefore pose as a limitation to the current study.

One final limitation of the current study was the task used to assess working memory functioning. The N-back task does not allow for the evaluation of specific working memory subprocesses and previous research has demonstrated oscillatory alterations within these subprocesses (Haenschel et al., 2009). Therefore, we were unable to determine if cannabis differentially modulated the encoding, maintenance, manipulation, or retrieval stages of memory. Additionally, repeated working memory and EEG testing has been shown to be influenced by within-subject variability. A recent study revealed that while the overall score, cortical activation, and alertness, showed long-term stability within subjects, these measures were sensitive at the level of the individual. Cognitive function generally varied due to
within-day fluctuations including alcohol, marijuana and caffeine use; these fluctuations were greatest when testing sessions took place in the afternoon (Gevins et al., 2012). While participants in the current study were instructed not to use alcohol, caffeine, or marijuana on the testing day, these factors as well as variability within testing times likely influence the current findings.

4.3 Conclusions

To our knowledge, this was the first study to address the complex relationship between cannabis, cognition, and cortical inhibition in cannabis dependent patients with schizophrenia using a within- and between-subjects longitudinal design. We hypothesized that patients would demonstrate reduced cortical inhibition and excitation indexed through SICI, LICI and ICF measures, as well as a shorter silent period, as assessed through CSP. We also hypothesized that patients would demonstrate poorer working memory performance compared to controls as well as excessive gamma oscillatory activity specifically in the frontal region in patients with schizophrenia. Finally, in regards to our longitudinal data, we hypothesized that following abstinence, both patients and controls would exhibit improved cortical inhibition and working memory performance, with the greatest improvements in patients.

However, contrary to previous findings (Fitzgerald et al., 2009; Wobrock et al., 2010) at baseline, cannabis dependent patients demonstrated significantly greater GABA<sub>B</sub> function, as measured by CSP. Additionally in the current study, no diagnostic differences were found on baseline measures of working memory accuracy or gamma oscillatory activity. When compared to a non-using sample, our current dependent controls performed worse on the 3-back condition of the N-back whereas cannabis dependent patients performed better than
non-using patients. Taken together, these findings would suggest that cannabis dependence might have more impairing effects, specifically on GABA\textsubscript{B} mediated inhibition, in controls compared to patients.

Given that cannabis reduces GABA function, and GABAergic neurotransmission underlies both cortical inhibition and cognition, it follows that cannabis dependence would lead to deficits in these domains. This pattern was clearly observed in our control group. However, this mechanism cannot account for our findings in cannabis dependent patients. Converging evidence suggests that cannabis-using patients may represent a subset of schizophrenia patients with better premorbid cognitive and social functioning, and therefore, potentially less impaired cortical inhibition. It is clear that continued research is necessary in order to better understand the mechanism underlying this common co-morbidity to work towards developing more specialized treatment approaches.

4.4 Future Directions

Given that this is the first study evaluating the effects of cannabis dependence and abstinence on cortical inhibition and working memory in patients with schizophrenia, it is clear that future investigation is needed in order to address the aforementioned limitations. Other than our CSP findings, the baseline analysis did not yield statistically significant differences between controls and patients, suggesting that continued research is needed in order to elucidate the potential differential effects of cannabis. This also highlights the heterogeneity of both schizophrenia (Davidson & McGlashan, 1997) and cannabis use (Iversen, 2003). Given the large \textit{interindividual} and \textit{intraindividual} variation over time in this population, much larger samples may be needed in order to elucidate group differences. Furthermore, future research would benefit from enlisting a true control group of non-using
patients or controls. This four-way comparison would provide additional insight into the differential effects of cannabis use on patients with schizophrenia and non-psychiatric controls.

The current study sought to investigate the effects of the cannabis-psychosis comorbidity primarily on GABAergic functioning, however, both cannabis and schizophrenia are known to influence a range of neurotransmitters, especially dopamine. For example, CB$_1$ activation through THC is known to enhance mesolimbic dopaminergic neurotransmission. Converging clinical and preclinical research has implicated this increase in dopamine in both the cognitive impairments (Pistis et al., 2002; Pistis et al., 2001) and reinforcing properties associated with cannabis use (Ameri, 1999; Bossong et al., 2009). While PET studies investigating the effects of chronic and recreational cannabis use exist, studies focusing on the long-term neurophysiological effects in abstinent users are limited. Thus, given the widespread neurochemical effects of both cannabis use and schizophrenia, in order to gain a true understanding of the neurophysiological effects in this population, future research should take into consideration additional neurotransmitter systems.

Similarly, future research should also focus on additional oscillatory frequencies including theta gamma coupling, which has also been implicated in working memory functioning (Canolty et al., 2006). There is a paucity of research investigating the functionality of theta-gamma coupling in patients with schizophrenia, and to our knowledge, this topic has not been investigated in cannabis users. In the current study, we chose the prefrontal cortex as the optimal site to investigate gamma oscillatory activity from, given its involvement in working memory processes. However, neuroimaging studies have shown that working memory functioning relies on a complex network spanning frontal and posterior
parietal areas (Honey et al., 2002). Research has suggested that the prefrontal cortex may be involved in retrieval whereas the posterior regions are associated with information storage (Curtis & D'Esposito, 2003). Unfortunately, the N-back task used in the current study does not allow for the investigation of the distinct subprocesses associated with working memory functioning. Taken together, this highlights the need for future research examining additional oscillatory frequencies spanning the frontal and parietal working memory networks. In order to better understand the exact influence of cannabis and schizophrenia on working memory, future studies should employ a memory task that allows for such assessments.

An additional and extremely important future direction would be to assess cortical inhibition from the DLPFC, given its direct involvement in the pathophysiology of schizophrenia (Barch et al., 2001) and evidence revealing eCB alterations in this region in patients (Dean et al., 2001). Moreover, many of the cognitive side effects associated with cannabis use are thought to be derived from CB1 receptors in the DLPFC. Recent technological advances have allowed for the combination of TMS with EEG. Using a pharmaco-TMS-EEG approach, researchers have been able to study the direct role of GABAergic neurotransmission through TMS-evoked EEG potentials (Premoli et al., 2014). Therefore, this technique would likely provide valuable insight into the mechanism underlying co-morbid cannabis use in patients with schizophrenia.

Future research would also benefit from employing longitudinal abstinence paradigms, which not only provide insight into the long-term neurophysiological effects of cannabis but also control for some of the confounding variables associated with between-subject study designs. Employing a longitudinal design may provide clarification as to whether the observed results were due to the residual effects of THC in the body, acute
cannabis withdrawal, long-term cannabis consumption, or premorbid neurodevelopmental dysfunction seen in patients with schizophrenia.

Furthermore, in order to understand its effects within complex co-morbid populations, we must first better understand the neurochemical and neurophysiological impact associated with cannabis use. Additionally, further research specifically focusing on the long-term effects of chronic use, and early initiation of cannabis use is needed as the clinical importance of cannabis research lies within this problematic pattern of use. Thus longitudinal research should also focus more so on naturalistic substance use in the general population rather than acute administration of THC using lab-based methodologies. While these naturalistic studies introduce variability, in terms of the strain and pattern of cannabis used as well as history of cannabis and other drug use, they also allow for more generalizable and relevant findings. This variability within the cannabis using population highlights the fact that cannabis research is still in its infancy and continued investigation is needed.

Elucidating the neurophysiological effects of cannabis dependence would provide novel and valuable insight into this common co-morbidity. This field would benefit from multidisciplinary research both neurophysiological and cognitive based, in order to ultimately catalyze future research regarding intervention and treatment strategies. It is evident that there is a need for biologically-based investigation of mechanisms underlying this common co-morbidity. As such, more funding is needed in order to allow for a more intense investigation in this area. While the findings from the current study are preliminary, they provide a first step towards better understanding the impact of cannabis on patients with schizophrenia. Whether continued research ultimately determines that cannabis use has more impairing neurophysiological effects on patients with schizophrenia or seemingly positive
effects, the findings will be of value. However, it is clear that given the widespread use of cannabis among patients with schizophrenia, the known negative effects on illness course and the lack of specific treatment approaches targeted at cannabis dependent patients, further investigation is of great importance.


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Sokolov, B. P. (1998). Expression of NMDAR1, GluR1, GluR7, and KA1 glutamate receptor mRNAs is decreased in frontal cortex of "neuroleptic-free" schizophrenics: evidence on reversible up-regulation by typical neuroleptics. J Neurochem, 71(6), 2454-2464.


## Appendix A: Telephone Screen

<table>
<thead>
<tr>
<th>Screen ID:</th>
<th>Screen date:</th>
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<tbody>
<tr>
<td>Interviewer’s Initials</td>
<td>Eligible Recontact</td>
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<tr>
<th></th>
<th>Y</th>
<th>N</th>
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<td></td>
<td>Y</td>
<td>N</td>
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Name (first/last): ____________________________ Phone no H/W/C.: ____________________________

Age: _______ (16 to 55) DOB (dd/m/yr): ____________________________ Gender: M (exclude females)

Referral Source: __________

**SMOKING:**

Do you smoke tobacco?  No (exclude), Yes.

On average, how many cigarettes do you smoke in a day/week? _______

**PSYCHIATRIC HISTORY: (Diagnosis of schizophrenia or schizoaffective disorder, currently in TX)**

Have you ever had any psychiatric treatment?  No Yes, currently

Diagnosis? ____________________ Clinician: ____________________ Program: __________

Last hospitalization for psychiatric symptoms? ____________________

Current Medications (dose and recent changes) ____________________

(Must be on stable antipsychotic dose for at least one month, if not, re-contact)

Have you ever had a heady injury with LOC?  No Yes details

Did you black-out? For how long? Did you need to be hospitalized? ____________________

Are you experiencing medical problems at this time?  No Yes details ____________________

Do you have any metal in your body?  No Yes details ____________________
CURRENT SUBSTANCE USE:

Marijuana: ........................................... No (exclude)  Yes, assess for current dependence:

Frequency and duration of use:_____________________

• substance is taken in larger amounts or over long period than intended

• persistent desire to quit or cut down

• majority of time spent involved in activities of obtaining/using/recovering

• social, occupational, recreational activities given up or reduced

• continued use despite physical and psychological problems

• tolerance: ↑ needed for same effect/ ↓ effect with same amount

• withdrawal

Yes to 3 of above symptoms  No (exclude),  Yes

Are you currently actively trying to quit marijuana? (treatment-seeking are not eligible and should be referred elsewhere)

No  Yes (exclude),

CURRENT/PAST SUBSTANCE USE:

Alcohol: .................................................. Current:_____________ (exclude: M>14/ wk)  Past: _____________

Cocaine: ............................................... Current: Y (exclude)  N  Past: ________________

Opiates (heroin, p-dope, percocets): .............. Current: Y (exclude)  N  Past: ________________

Methadone: ............................................. Current: Y (exclude)  N  Past: ________________

“Pills” (valium, xanax, etc): ......................... Current: Y (exclude)  N  Past: ________________

Other (acid, speed, illy, etc): ....................... Current Y (exclude)  N  Past: ________________

Cannabis Abstinence Study Eligibility:

No  Yes  Yes, possibly  Availability: _

Would you be willing to quite marijuana for a one-month period?

IN-PERSON appointment SCHEDULED:  No  Yes  TBA (pending)

If Yes: Appointment date, time, RA: ________________________________

Consent to contact for participation in future studies or add to Sz research registry
Appendix B: Study Consent

STUDY INFORMATION AND CONSENT FORM:

Effects of Cannabis Abstinence on Neurocognition in Schizophrenia: Neurophysiology Assessment

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Contact Phone Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tony P. George, MD, FRCPC</td>
<td>416-535-8501 x 34544</td>
</tr>
<tr>
<td>Zafiris J. Daskalakis, MD, PhD, FRCPC</td>
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</tr>
<tr>
<td>Rachel A. Rabin, MSc</td>
<td>416-535-8501 x 36115</td>
</tr>
<tr>
<td>Mera S. Barr, PhD</td>
<td>416-535-8501 x 33095</td>
</tr>
</tbody>
</table>

Study Purpose:

The use of cannabis influences the brain’s ability to concentrate, pay attention, reason, and remember. Collectively these are known as cognitive functions. How cannabis affects these cognitive functions are still unclear.

Cannabis may influence brain mechanisms called cortical inhibition and plasticity, which in turn, influences cognitive function. Cortical inhibition is a brain process that is designed to filter excessive information in our environment in order to process information efficiently. Plasticity is involved in learning and memory and. It has been discovered that cortical inhibition and plasticity may impaired in the brains of people who use cannabis. Cortical inhibition and plasticity is also impaired in patients with schizophrenia who suffer from cognitive impairment as part of their illness.

Transcranial magnetic stimulation (TMS) is a method used to measure brain inhibition. TMS excites nerves over the area of the brain that is responsible for thinking and planning. When the nerves are stimulated, this causes brain activity to change, which will be recorded with electromyography (EMG) and during electroencephalography (EEG), which will be analyzed later.
The purpose of this study is to measure cortical inhibition, plasticity and cognitive function in patients and control participants who are cannabis dependent. These measures will then be evaluated during a one month period of abstinence. We hope that this study will help us understand the interaction between cannabis, cognition, and schizophrenia. This is not part of any treatment plan.

Procedures:

You are being asked to provide informed consent. After reading through this form, you will be given a chance to ask questions. All study procedures will be completed at Dr. George’s BACDRL lab (1st floor, 33 Russell Street, Toronto, ON, Room 1910A) and at the Temerty Centre for Therapeutic Brain Intervention (1001 Queen Street West, Unit 4-1, Room 124) at the Centre for Addiction and Mental Health.

The study will take place over a 1-month period + a one-month follow-up, where you will be asked to come into the lab. These sessions will either take place on the same day as primary assessments or can be done within the same week on a separate day.

The assessments will be conducted by Ms Michelle Goodman, Ms. Rachel Rabin and Dr. Mera Barr. Ms Goodman and Rabin are graduate students in the Institute of Medical Science at the University of Toronto and the Centre for Addiction and Mental Health, Schizophrenia Program, and this study is part of their graduate research program. Rachel Rabin will lead the project and be responsible for recruitment, enrolment and screening of participants, as well as assessments and cognitive testing. Ms. Michelle Goodman will be responsible for the neurophysiological assessment conducted at the Temerty Centre. Dr. Mera Barr is an Independent Scientist in the Schizophrenia Division of the Complex Mental Illness Program at CAMH. Dr. Mera Barr will oversee the assessment of cortical inhibition and plasticity with TMS and measure the memory task with electroencephalography (EEG). Drs. George and Daskalakis are research psychiatrists in the CAMH Schizophrenia Division who act as supervising and medically qualified investigators for this study.

DT-MRI

The sessions to measure cortical inhibition in your brain will be conducted at baseline prior to the initiation of cannabis abstinence, once a week for 4 weeks (during the one month abstinence), and one month post abstinence.

- The MRI will be done at the Centre for Addiction and Mental Health. The MRI scanning is a technique of taking pictures of the brain that gives information on brain structure and brain function.
- Before the scan begins, you will be asked to remove all metal that you are wearing because MRI generates images using a strong magnetic field and this can damage some electrical devices.
- You will then be asked to lie on a padded bed that will be moved into a tunnel-like
machine for the MRI scan. The tunnel is not very large, and the study requires you to lie still in the scanner.

- You should try to remain as still as possible during the scan. Movements will not be dangerous to you in any way, but would blur the picture of your brain.
- You will be in the scanner for about 60 minutes.
- You might hear moderately loud knocking or beeping during the scan when the MRI machine is in operation. These sounds reflect the normal functioning of the MRI.
- MRI scanning is not associated with any known risks to your health, and there is no proof that there will be short-term or long-term side effects.
- This will be done before and after 28 days of abstinence.

**TMS**

TMS sessions will measure cortical inhibition and plasticity in your brain. At baseline and following cannabis abstinence, we will measure cortical inhibition and plasticity using TMS and EEG. Once a week during the one month abstinence (4 testing sessions) and 1 month post abstinence we will conduct the TMS session without EEG. The TMS and EEG sessions at baseline and following cannabis abstinence will take approximately 8 hours. The weekly TMS sessions and one month follow up will take approximately 30 minutes.

**Cortical Inhibition**

- We will attach soft foam electrodes to the skin surface over your hand muscles, and then connect these to a recorder that will record the activity of your hand muscles (for the first part, then TMS will be applied to the front of your head)
- A magnetic coil will be held on the surface of your scalp
- To obtain brain inhibition, different modes of TMS will be applied to the head surface and EMG will be recorded from your hand muscle.
- When the magnetic stimulation is applied you will feel a twitch or small movement in your hand but no pain.
- There are many pulses but each pulse is very short and not too close to the next.

**Plasticity**

- This set up will be the same as measuring cortical inhibition but with an additional electrode placed on your right wrist that will deliver low level electrical stimuli prior to the delivery of the TMS pulse
- There will be several pulses with TMS alone and TMS combined with the electrical stimulus
- This will take approximately 3-4 hours.

**EEG**
The sessions to measure TMS cortical inhibition, working memory, and mismatch negativity will be conducted at baseline prior to the initiation of cannabis abstinence and week 4 of abstinence.

- EEG or electroencephalography is a measurement of the electrical activity of the brain.
- We will use this to look at brain activity during TMS brain stimulation.
- You will be seated in a comfortable chair and will have on a cap containing many electrodes that record your brain activity. It takes approximately 30 minutes to put on the cap and to prepare for recording.
- There is gel on the inside of the cap that may be sticky. Shampoo will be provided after the testing session to wash your hair.
- You will be asked to rest comfortably while the TMS is applied and the computer is recording information. The combination of TMS and EEG will take approximately 4.5 hours.

Working Memory Task

- We will use this to look at brain activity while at rest and during a working memory task.
- You will be seated in a comfortable chair and will have on a cap containing many recorders that record your brain activity. It takes approximately 15 minutes to put on the cap and get it ready for recording.
- There is gel on the inside of the cap that may be sticky; you will be allowed to rinse it out after the test.
- The recorders are attached to a computer to record the information.
- This will last approximately 1.5 hours.

Mismatch Negativity Task (MMN)

- We will use this to look at brain activity that takes place when you are not directly paying attention to auditory stimuli.
- You will be seated in a comfortable chair with the EEG cap and two foam earphones placed in the ears.
- Auditory tones will be played in both ears while you watch a silent cartoon video.
- This will last approximately 45 minutes.

Are there any side effects or pain associated with TMS, EEG, or MRI?

There may be some mild side effects with transcranial magnetic stimulation. At certain positions on the head, the stimulation may cause your eyes to blink or a brief contraction of the scalp, neck, trunk or upper arm muscles. You may find these contractions annoying, but they should not be painful. Some people may experience mild headache or shoulder stiffness after testing, however, these symptoms will usually go away in time. Acetaminophen
(Tylenol) is effective in treating these side effects. If you have further concerns you may contact your study doctor at any time. The magnetic stimulator makes a clicking noise when it stimulates. Since this may be distracting, you will be offered ear-plugs which mute the sound. Magnetic brain stimulation has been used on thousands of individuals in the United States, Canada and Europe over several years without any serious problems. There are no serious side effects for EEG. Some people report a headache following the procedure but this is only due to the pressure of wearing a cap and not the procedure itself. MRI is a non-invasive procedure. Certain items such as a pacemaker and having surgical staples or other ferrous metal in or near the head are dangerous with an MRI. For this reason you will be screened using the precautionary screen for these items designed by the MRI Unit at CAMH.

**Risks**

You should be aware that the magnetic fields generated by the stimulator may damage magnetic cards, watches and some electrical devices. *Please remove any such items before testing.*

Exposure to magnetic stimulation or any strong magnetic field is not permitted in people who have a pacemaker, an implanted medication pump, a metal plate in the skull, or metal objects inside the eye or skull (for example, after brain surgery or a shrapnel wound). *Please inform the investigators if you might have any of these.*

Magnetic brain stimulation has caused brief epileptic seizures in a few patients with stroke and epilepsy out of thousands of people tested. This has never happened with the form of TMS you will be receiving. Patients with epilepsy and patients with stroke can have an increased risk of seizures. Therefore, there is a very small possibility that magnetic stimulation may cause seizures in persons with a heightened risk. The investigators cannot promise that this will never happen in the future; however, the probability of this occurring in the experiment is low. As we have stated above, these tests have been used in thousands of healthy volunteers all over the world and there is no report of a seizure in healthy people.

<table>
<thead>
<tr>
<th><strong>Common</strong></th>
<th><strong>Remote</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache and Scalp pain</td>
<td>Effects of Hearing (earplugs are worn to prevent this)</td>
</tr>
<tr>
<td>Tingling sensation and/or heat at coil location</td>
<td>Syncope</td>
</tr>
<tr>
<td></td>
<td>Seizure (no reports in current literature during paired-pulse TMS)</td>
</tr>
</tbody>
</table>
You might feel slight fatigue during the testing sessions. While a cannabis withdrawal syndrome has been reported it does not include significant physical, medical, or psychiatric problems. You may experience irritability, anger, depression, difficulty sleeping, craving, and decreased appetite. Headaches, physical tension, sweating, stomach pain, and general physical discomfort have also been observed during cannabis withdrawal, but are less common.

**Benefits:**

The information you provide will improve our knowledge about the effects of cannabis abstinence in individuals with schizophrenia, and might encourage you to want to quit using cannabis.

**Voluntary Participation & Subject Obligations:**

You are free to choose not to participate, and may withdraw from this study at any time. If you withdraw, it will not affect your ability to receive treatment at CAMH. Study staff/investigators may, at their discretion, end your participation in this study at any time. If you are an existing CAMH client, you will also be asked to give the research team permission to access your medical records for the purpose of confirming your medication and treatment status.

**Study Provisions:**

You will be paid for attending and completion of each study visit at a rate of $10/ hour for a total of 9 hours. If all visits are attended for a total of $220.
Payment Schedule: $10/hour for a total of $200

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Assessment Length (UP TO; h)</th>
<th>Assessment Completion (UP TO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline: PAS, TMS-EEG, Working Memory, MMN, and fMRI</td>
<td>9 hours</td>
<td>$90</td>
</tr>
<tr>
<td>Wk 1 TMS</td>
<td>1 hour</td>
<td>$10</td>
</tr>
<tr>
<td>Wk 2 TMS</td>
<td>1 hour</td>
<td>$10</td>
</tr>
<tr>
<td>Wk 3 TMS</td>
<td>1 hour</td>
<td>$10</td>
</tr>
<tr>
<td>Wk 4: PAS TMS-EEG Working Memory, MMN, and fMRI</td>
<td>9 hours</td>
<td>$90</td>
</tr>
<tr>
<td>Wk 8: TMS</td>
<td>1 hour</td>
<td>$10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>22 hours</td>
<td>$220</td>
</tr>
</tbody>
</table>

Confidentiality:

If you decide to take part in this research study, you will be required to answer some questions about your drug use and problems you may be having relating to drug use. Your answers to these questions, as well as other data collected will only be used by the study investigators and their designates and will remain confidential to the extent permitted by law.

- As part of continuing review of the research, your study records may be assessed on behalf of the Research Ethics Board. A person from the research ethics team may contact you to ask you questions about the research study and your consent to participate. The person assessing your file or contacting you must maintain your confidentiality to the extent permitted by law.
- In accordance with federal requirements, CAMH will maintain archived study records for 10 years. However, those documents that contain personal identifiers (i.e. consent forms) will be stored separately from data files.
- General results of this study might be published, but will not identify you by name.
As part of the Research Services Quality Assurance Program, this study may be monitored and/or audited by a member of the Quality Assurance Team. Your research records and CAMH records may be reviewed during which confidentiality will be maintained as per CAMH policies and extent permitted by law.

Contact: If you have any further questions or desire further information about this study, you may contact Rachel Rabin at 416-535-8501 x 36115, Dr. Mera Barr at 416-535-8501 x 33095 or the Principal Investigator Dr. Tony George at 416-535-8501 x 34544. If you have any questions about your rights as a study participant, you may contact Dr. Padraig Darby, Chair of the Research Ethics Board, Centre for Addiction & Mental Health, at 416-535-8501 (x36876).
AGREEMENT TO PARTICIPATE

I, ___________________________________ have read (or had read to me) the information form for the study named *Effects of Cannabis Abstinence on Neurophysiology in Schizophrenia: Neurophysiology Assessment*. I understand that my role is that of a participant in this study. I have been given an opportunity to ask questions about this study. Any questions that I have had, have been answered to my satisfaction, so that I now understand the study procedures, the potential risks of participating, and my right to the confidential treatment of the information that is collected about me. I also understand that my participation in this study is entirely voluntary, and that I may refuse to participate or withdraw from the study at any time, without any consequences for my continuing care. By signing this consent form, I do not waive any of my legal rights nor relieve the investigators/institution from legal responsibilities. If I have any questions about my rights as a study participant, I may contact Dr. Padraig Darby, Chair of the Research Ethics Board, Centre for Addiction and Mental Health, at 416-535 8501, extension 6876.

I have received a copy of this consent form for my own record.

Participant’s Initials:_______

<table>
<thead>
<tr>
<th>Participant Name:</th>
<th>Person who conducted informed consent discussion:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Print name</td>
<td>Print name</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Signature of Participant</td>
<td>Signature of Witness</td>
</tr>
<tr>
<td>Date:</td>
<td></td>
</tr>
</tbody>
</table>
Appendix C: Case Report Form

Effects of Cannabis Abstinence on Neurocognition in Schizophrenia: Neurophysiology Assessment

Subject ID: ___________________________

Testing Completed by: __________________

Time Point: Week 0

Date: ________________________________

Time of Last cannabis smoked: _________ AM  PM

Testing Time Start: _____________________ AM  PM

Testing Time End: ______________________ AM  PM

Without Cap

Resting Motor Threshold: ______________

1 mV Peak to Peak: ______________

SICI: ____________________________________________

LICI: ____________________________________________

CSP: ____________________________________________

With Cap

Resting Motor Threshold: ______________

1 mV Peak to Peak: ______________

Rest:

LICI Motor Single: ____________________________________________

LICI Motor Paired: ____________________________________________

ICF Motor Paired: ____________________________________________

LICI DLPFC Single: ____________________________________________

LICI DLPFC Paired: ____________________________________________

ICF DLPFC Paired: ____________________________________________
Nback 1-back: ___________________________________________________________

3-back: ___________________________________________________________

Time Point: Week 0

Comments:
____________________________________________
____________________________________________
_________________________________________________________________________________
_________________________________________________________________________________
____________________________________________